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NEW RESULTS ON THE HISTORY OF CULTIVATED CEREALS

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1. INTRODUCTION

(*a*) *Gene centres*

In speaking of the origin of cultivated plants, two very different branches of scientific research are concerned—Botany and the History of Human Culture. Both meet at their origin—since the word "culture" in any language has, at its root, the meaning of tilling the ground in order to get edible crops from it. So the very expression "cultivated plants" signifies a wider view, embracing on one side the natural sciences, Botany, Agriculture and Genetics, on the other, Archæology, Pre-History and Early History of Agriculture. These two different sides of research, each with their own methods, have co-operated to the solution of a common problem; there lies the special attraction of these studies.

Alphonse de Candolle, the pioneer of the subject, was driven by an historical, an evolutionary interest. When trying to understand the history of the plant kingdom as a whole—its evolution in space and time—he found the cultivated plants to be an example, a model in miniature, for the whole process. Derived from the wild flora, the cultivated plants had developed to the great variability and high status of to-day in the restricted time of some thousands of years (since the appearance of man) and in an area, the geological change of which, during the period, is fairly well known. Their evolution might thus elucidate the transformation of the plant kingdom in a

much longer period, and under incompletely known geological, climatic and edaphic changes—a transformation ending in the admirable adaptation to their habitat we meet with to-day.

Since that time the two great papers of de Candolle on the origin of cultivated plants have been the standard work in this question, and it was not until 1927 that a new period of research was inaugurated by Vavilov's theory of gene centres as centres of origin of cultivated plants. Crop plants which had been neglected in botanical research up to then, were lifted to general interest by the new taxonomic and phytogeographical methods he evolved and their connection with genetical problems. Much special work on single crops has been done since that time and such investigations have contributed to a better understanding of the evolution of plants in general, as well as to the theoretical basis of plant and animal breeding.

Vavilov's theory, though accepted with acclamation from the botanical and the agricultural sides, has not remained without criticism in some details. In 1932 I gave a first account of the international contributions to the problem, and pointed out some discrepancies, concerning the origin of our chief cereals. Vavilov did not admit the direct descent of barley and wheat from the wild grasses *Hordeum spontaneum* or *Triticum dicoccoides* although he accepted the descent of rye and oats from *Secale afghanicum* and *Avena fatua*.

On behalf of the abstract part of the theory, Turesson entered into discussion as a critic. Later on it was taken up by the present writer and the late R. Freisleben. In 1943 I gave a summary of the general progress in this field with a special discussion on the phylogeny of wheat and barley. Darlington, in the introduction to the *Chromosome Atlas of Cultivated Plants* in 1945 has connected some of the new results with the chief idea of Vavilov's theory and their practical consequences for plant breeding. The intention here is to give an account of the results which German science has contributed to some main questions during a period of scientific isolation, and to connect them with the international work of the last decade—following in substance a paper read in Stockholm at the 7th International Congress of Botany in 1950.

(*b*) *Mode of evolution*

For a better understanding some introductory remarks on the mode of evolution of the cereals dealt with in this paper may be given :—

We regard as ancestors of our cereals some wild grasses, morphologically very similar to them. These are distinguished from their other wild relatives by large mealy grains, but resemble them in having a brittle axis (rachis) adapted to self-propagation. The large grain of the wild type may have invited man to collect it for food and to sow it around his settlement. The tough axis and other qualities of the cereals are supposed to have arisen by gene mutation.

Self-propagation and self-protection once lost and being of no use in cultivation, the new cultivated type is fixed by unconscious and conscious selection. All " cereals " have a tough axis.

TABLE 1

Triticum L. Weizen, Wheat

	Monococca Flaksb. Einkorn series $2n = 14 = AA$ (Diploidea Fl.)	*Dicoccoidea* Fl. Emmer series $2n = 28 = AABB$ (Tetraploidea Fl.)	*Speltoidea* Fl. Dinkel series $2n = 42 = AABBDD$ (Hexaploidea Fl.)
Wild hulled, brittle	*boeoticum* Boiss. (= *aegilopoides*) wild small spelt (Balkans, Anatolia)	*dicoccoides* Kcke wild emmer (Palestine to Transcaucasia) *Timopheevi* Zh. $2n = 28 = AAGG$ (Transcaucasia)	none
Cultivated (not brittle) — Hulled type Spelzweizen	*monococcum* L. Einkorn, small spelt (relic-cultures : Europe, Anatolia, N. Africa)	*dicoccum* Schübl. Emmer (relic-cultures : Europe, S.W. Asia, India)	*Spelta* L. Spelz, Dinkel ; Spelt wheat (Rhine-Valley, Switzerland to Belgium) *macha* Dek. et Men. (Transcaucasia)
Cultivated (not brittle) — Naked type Nacktweizen	none	*durum* Desf. Hartweizen, macaroni-wheat (Mediterranean - Turkestan, Steppes of S.E. Europe, N. America) *turgidum* L. *polonicum* L. *carthlicum* Nevski *turanicum* Jak. (Mediterranean, Near East, Abyssinia)	*aestivum* L. (= *vulgare*) Saatweizen, Bread wheat, soft wheat (pan-temperate) (incl. *compactum*, Binkelweizen, Club wheat) *sphaerococcum* Perc. Kugelweizen, Indian dwarf wheat (N.W. India)

The loss of self-protection goes different ways in wheat and barley. In *wheat* (and in rye) the wild grass has tightly closed glumes, keeping the grain " hulled " when thrashed—" Spelzweizen." Some of the cultivated wheats retain this primitive feature (small spelt, emmer, spelt). A further mutation leads to loosely closed glumes, which give a naked corn when thrashed—" Nacktweizen." The naked wheats are the important crops.

In *barley* lemma and palea adhere to the grain, in the wild type as well as in most of the cultivated barleys—" Spelzgersten." Naked barley is a mutation within the cultivated type, isolated by selection.

In *Hordeum* a morphological development on a reduction-line leads from the primitive fully-developed ear with two rows of fertile triplets, forming six rows of grains—Section *Polysticha* (many-rowed barleys)—to forms with highly reduced lateral florets. The remaining middle

florets of the triplets form two rows of grains in the ripe ear—Section *Disticha* (two-rowed barleys). The difference between six- and four-rowed barley in the *Polysticha* is but a difference of density of the ear and has no taxonomical importance.

In both sections the reduction of the lateral florets continues. *H. deficiens* in the *Disticha*, an endemic of Abyssinia, has completely reduced lateral florets. *H. intermedium*, in the *Polysticha*, an endemic of Tibet and East China, is a potentially six-rowed barley with lateral florets of reduced fertility and grain size and often with changed morphology. Recently I detected the brittle prototype of this form in East Tibetan seed material and called it *H. paradoxon ;* it may be understood (like *H. spontaneum*) as a direct mutant from *H. agriocrithon*. Either by mutation or by hybridisation with *H. vulgare* it gave rise to the tough-eared *H. intermedium*.

TABLE 2

Hordeum L. Sectio *Cerealia* And. Barley, Gerste

	Series *Polysticha* Many-rowed		Series *Disticha* Two-rowed
Wild brittle, hulled	all florets alike	lateral florets of different morphology	lateral florets reduced
	agriocrithon Åberg * (Tibet)	*paradoxon* Schie.* (Tibet)	*spontaneum* Koch * lateral fl. male (Near East, west of Hindukush)
Cultivated tough rachis hulled or naked	*vulgare* L. 6-*a*. four-rowed barley (pan-temperate)	*intermedium* Carl.* (Tibet-China)	*distichum* L. lat. fl. sexless to male (Near East, west of Hindu Kush ; recently pan-temperate)
	mut. nudum naked barley, Nackt-gerste (pan-temperate)		*deficiens* Steud. lat. fl. reduced to traces Fehlgerste * (Abyssinia)

2. NEW MATERIAL FROM COLLECTIONS

These contributions are based on new collections of wheat and barley brought home by a series of scientific expeditions to the mountains of High Asia (Schiemann 1943, Tab. 9) :—

1935. " Deutsche Hindukusch Expedition " under Scheibe (D.H.E.).
1937. " Nanga Parbat Expedition " with Troll as botanist.
1937-38. " India-Nepal Expedition " of Herrlich.
1939. " Tibet Expedition " under Schaefer.

* No English popular name.

Besides providing valuable material for practical purposes, these collections have been of importance for two questions that interest us here: the origin and evolution of barley and the theory of gene-centres as developed by Vavilov.

The rich material of the German collections has been analysed for wheat by Maria Lange de la Camp and for barley by R. Freisleben.

(a) Wheat

The Hindu Kush expedition, following in part Vavilov's route of 1924, was the first to explore the area of Chitral and Nuristan. In conformity with Vavilov's statement for the Hindu Kush-Himalayan centre of hexaploid wheats, it widened our knowledge on the geographical area and the variability of this group. A thorough study of the racial composition of the human tribes—following Vavilov's aims here too—gave an explanation for the combination in the Hindu Kush of forms of both subspecies of *T. æstivum* distinguished by Flaksberger: ssp. *indo-europæum* and ssp. *irano-asiaticum*. The diversity found here may (beside purely natural influences) greatly depend upon migration and mixture of peoples and tribes in olden times. The Indo-European wheats and the Indian *sphærococcum* entering the area of ssp. *irano-asiaticum* in these countries, Vavilov's conception is comprehensible, that the Middle Asiatic centre embraces the whole of the genes of hexaploid wheats.

(b) Barley

In 1934 Harry Smith of Uppsala brought home from Tibet—from Taofu near to the frontier of Szechuan, a six-rowed brittle hulled barley as weed among purely naked cultivated barley, described by Åberg in 1938 as *Hordeum agriocrithon*. This plant proved to be the long-looked-for wild form of the presupposed primitive type of barleys, the six-rowed type. Until then, only two-rowed brittle barleys were known, which morphologically must be looked on as a reduction-form. Schaefer on his expedition to Tibet in 1938 brought home a second find of this type from Lhasa 1200 km. south-west of Taofu, described by Freisleben. Since this was not collected from the field, but brought home as five specimens of pure seed from the market, Freisleben, though attributing it to *H. agriocrithon* Åberg doubted of its being a really wild grass. Recently I detected a third specimen in the cereals of a Tibet expedition of the British Museum *; this time as a weed again in naked barley.

The second result in barley, established by the German expeditions and completed by literature studies of Freisleben, is the assertion, that in the whole agricultural area east of the Hindu Kush and the

* The seed has been kindly delivered to me for investigation, and I wish to express my gratitude to the British Museum here (*cf.* Schiemann, 1951).

bordering mountains, only many-rowed barleys are cultivated—this holds good for India, Tibet, China, Manchuria and Japan. In India two-rowed barleys were introduced from Europe later than 1872, in Japan about 1868. The Hindu Kush expedition showed that two-rowed cultivated forms begin only in the area of *H. spontaneum* and show the same variability as the many-rowed forms of the area concerned. Scheibe picked up a natural hybrid between *H. spontaneum* and a four-rowed tough barley in North Turkistan.

On these facts Freisleben, partly in accordance with Åberg, developed the hypothesis, that two-rowed cultivated barleys originated merely at the contact with six-rowed cultivated barleys, penetrating—in prehistoric times—from the Far East into the area of *H. spontaneum*. They arose here as hybrids and were stabilised by selection, after which they spread together with the many-rowed forms, their ancestors, repeating the variability of these.

This theory helps to elucidate the occurrence side-by-side of two- and six-rowed, of hulled and naked barleys in agriculture since the European Neolithic Age, as well as the well-known uniformity, in spite of local peculiarities, of the barleys of the whole world. A fully continuous variability is guaranteed by the low and constant chromosome set of seven haploid chromosomes in all cultivated barleys and their ancestors. Whilst two-rowed wild barley must have come from a six-rowed wild form, Freisleben, contrary to Åberg, does not believe two-rowed cultivated barleys to be directly derived from six-rowed cultivated forms (by mutation), because the appearance of two-rowed cultivated barley is so strictly connected with the eastern border of *H. spontaneum ;* this would mean that all two-rowed cultivated barleys would primarily be of hybrid origin—their centre of origin then being the area of *H. spontaneum* in the Near East (Anatolia-Transcaucasia-Persia).

3. CRITICISM OF THE GENE-CENTRE THEORY OF VAVILOV

New light came from these area studies on the theory of gene centres. Åberg's discovery of six-rowed wild barley in Tibet and the exclusive culture of six-rowed barleys in East Asia were in agreement with Vavilov's conception, that the centre of diversity, the gene-centre, is at the same time the centre of origin for this special cultivated plant. As we saw, the Near East is such a centre for two-rowed barley.

But the question arose again, whether Abyssinia had to be regarded as a second independent centre of origin for—as Vavilov thought—especially hulled barleys, six- and two-rowed again.

Vavilov (1926), pointing out that *H. spontaneum* as well as *T. dicoccoides* have not been found either in Abyssinia or in the Himalayan centre, concluded that these species could not be the ancestors of cultivated cereals. He gives some further "proofs" of this view, viz. the restricted variability of these wild, large-grained grasses, cytological and fertility disturbances in hybrids with cultivated forms

and their ecologically different habitats. In 1932 (p. 393) and more explicitly in 1943 (p. 452 ff 506 ff), I showed these objections not to be tenable against older and more recent observations. And there was another difficulty, which arose from the existence of the wild two-rowed barley *H. spontaneum* in the Near East, half-way between Vavilov's two centres in East Asia and Abyssinia. So I suggested a direct descent of two-rowed barley from the different types of *H. spontaneum* of Transcaucasia and Persia, known from Vavilov's collections, which stand nearer to the cultivated two-rowed hulled forms than the spontaneum-type of Koch from Palestine.

Freisleben assumes that, owing to their hybrid origin, two-rowed cultivated barleys are of necessity mixed with many-rowed forms of similar type in Transcaucasia which therefore manifests itself as a third centre of diversity. Of these three, the Abyssinian one has to be regarded as a secondary centre of diversity, where two- and six-rowed barleys reaching the highlands of Abyssinia together, over Syria-Egypt or may be over Yemen-Eritrea, developed a new variability—in the same place where, according to Vavilov, tetraploid wheats also had a centre of diversity.

How then, if they are not centres of *origin*, are the Hindu Kush centre of hexaploid wheats and the Abyssinian centre of barley to be understood ?

(*a*) *Biological understanding of the gene-centres in mountains*

Since Vavilov lays stress on these centres being regularly situated in mountains, the great variability in such areas must be understood biologically. The question arises ; what makes these areas rich in gene differences ? Mutation experiments have shown that temperature shocks, which easily are realised in high mountains, cause a higher mutation-rate. Combination by free crossing, added to this, may well be responsible for the increase of variability in high mountains.

But what is the role of the second factor of evolution we know of, selection ? Here F. v. Wettstein in collaboration with K. Pirschle began to attack the problem of gene-centres experimentally. Working in Dahlem with climate chambers, adjusted to graded dosages of light, temperature, humidity and length of day, the first results showed the effect of ultra-violet radiation in different plant species, in different genera, collected partly in high mountains, partly in the plains. Ultra-violet light, and here the *medium* wave-lengths of 2700-3200 Ångstrom units, caused a change towards stunted forms, such as are characteristic of high mountains. The taller and more slender types were eliminated. What remained, proved to be genetically different from the non-radiated material. These results, demonstrating the selective influence of the habitat, may contribute to the understanding of certain endemic types in high mountain districts.*

* The experiments were abruptly cut off by the unexpected death of both investigators, of F. v. Wetterstein in 1945 and of Pirschle in the last days of the war. It will be necessary to proceed further on these lines.

(b) *The identity of centre of origin with the centre of diversity*

The question whether there is or is not identity between centre of origin and centre of diversity cannot be answered without entering into ethnological problems—concerning the beginning of agriculture and its propagation over the Old World.

According to Vavilov, Abyssinia, as we have seen, is the centre of origin of the tetraploid wheats and of two-rowed hulled barleys ; Middle Asia (the Hindu Kush area) of hexaploid wheats. Both areas are devoid of wild *Triticum* or *Hordeum*. But all investigators, including Vavilov himself in his later publications, have proved the area of Transcaucasia from the eastern parts of Asia Minor to the west of Persia and to the great deserts of North Syria in the south, to be enormously rich in wheat forms, not only varietal, but also specific, the greatest diversity being in the tetraploids. This area contains the wild emmer *T. dicoccoides*, and since there is no biological necessity to deny the phylogenetic step from *T. dicoccoides* to cultivated emmer, as is shown above (p. 311) we can ask whether historic and prehistoric dates agree with this view. This is indeed so.

In the whole area there is indeed, as Vavilov said, no *T. dicoccum* to-day except for some relic local areas in Transcaucasia and North-West Persia. But there is no doubt that emmer was the chief wheat cultivated from the very beginning of agriculture to the Roman Empire, in the whole of the Near East, Egypt, and the Mediterranean. This area includes the range of *T. dicoccoides* and provides geographical, ecological and ethnographical possibilities suitable for the transition from this wild grass with large grains, growing abundantly, adapted to natural propagation, into a " cereal " with tough rachis which man harvests and stores for new sowing in the next season. This same area we found to be the centre of origin of two-rowed barley.

Barley and emmer, being the first cereals cultivated, must have been brought to Abyssinia as plants already well adapted to cultivation by man. In Abyssinia, migrating peoples and with them their food plants, were arrested by the double barrier of the high mountains and the equator, both, we may presume, factors acting as promotors of mutations. These mutations would accumulate, combine by hybridisation and, since the tribes inhabiting Abyssinia have preserved —even to the present time—primitive methods of agriculture, selection would be practised more for simple yield than for morphological or physiological purity. I have called such a centre of diversity an *accumulation centre* (*Stauungszentrum*). It will be the richer in endemics the more it is isolated from human traffic. In this way Abyssinia became a secondary centre of diversity for two- and six-rowed barleys as well as for the tetraploid naked wheats, the origin of which may be referred to the Near East, from Transcaucasia to the Eastern Mediterranean Coasts.

This theory fits the Himalayan centre of diversity of hexaploid

wheats as well. Their origin as amphidiploids of a tetraploid wheat crossed with an *Ægilops*, which had long been suggested on behalf of the D-genome in *Æ. cylindrica*, has gained new probability from McFadden and Sears' synthesis of *T. Spelta* as an amphidiploid from *T. dicoccoides* × *Ægilops squarrosa* with $n = 7$ = D-genome. This supposed cross must have occurred in the common area of the two parents, which points again to the Transcaucasian centre.

From here the hexaploid wheats with the compact type as first representative must have spread westward in the Neolithic Age, while the lax forms of *T. æstivum* began to be of importance during the Roman Empire. On their way to the East, in the high Hindu Kush, the situation would be about the same as for the tetraploid wheats and the barleys in Abyssinia : extreme habitats, spontaneous hybridisation, favoured by a rather high degree of open flowering (according to observations of the D.H.E.), lacking selection in consequence of a primitive agriculture, are apt to accumulate mutations and combinations and thus form a centre of diversity, rather far from the area of origin. That fewer endemics are found here than in Abyssinia, might be explicable by the fact that these valleys, notwithstanding their great ethnographical as well as botanical diversity, have not been such a cul-de-sac as the Abyssinian highland. High passes have always allowed a certain amount of traffic—people have been in contact towards east and west and may have exchanged men and their goods and food plants.

No diversity has been found in barleys here. The eastern centre of six-rowed barleys being separated by the great deserts of Gobi, only few advancing forms have reached the Hindu Kush.

Nothing new has been found concerning *T. monococcum* and its ancestor *T. bœoticum* Boissier (= *T. ægilopoides* (Link) Bal.). Its centre of origin, Anatolia, coincides with its centre of diversity in the sense of Vavilov.

(c) *The importance of Vavilov's work*

These deductions lead to the conclusion that Vavilov's theory of gene-centres in so far as it assumes centres of diversity of cultivated plants to be centres of their origin, though valid in many cases, is not exclusively so. It holds good for *T. monococcum* with its centre in Anatolia, for the six-rowed barleys in East Asia, most probably also for *T. dicoccum* in Transcaucasia. But there are other centres of diversity—they may be called secondary centres. These are formed far from the place of origin as we showed to be the case for two- and six-rowed barleys in Abyssinia or hexaploid wheats in Middle Asia, and for them different causes can be alleged.

This critique of Vavilov's theory can in no way reduce his merit for the science of agriculture in theory and practice. He showed beyond doubt that there are centres of diversity for the single crops in rather few distinct parts of the whole area of agriculture. He

inaugurated a period of collecting expeditions which enriched our knowledge of crop varieties, and by this the raw material for plant breeding, immensely. His methods led to the introduction of genetics and cytology into the study of crop plants. By his introduction of the wild relatives in these analyses, the study of cultivated plants, so long neglected in general botany, was reunited with it. Having stagnated since de Candolle, it began to flourish again and to make use of new rich material as well as new methods.

4. ORIGIN OF THE HEXAPLOID WHEATS

Since the end of the war a new problem has arisen with the synthesis we mentioned already of *T. Spelta* by McFadden and Sears as an amphidiploid of *T. dicoccoides* × *Ægilops squarrosa*. With its definite introduction of the D-genome into the hexaploid wheats it has disproved decisively the hypothesis of Bertsch (still repeated in 1949 and 1950) which tries to build up *T. compactum* as an amphidiploid of *T. monococcum* × *T. dicoccum*. This would mean a composition merely of the genomes A and B, and give no explanation for the new characters of the hexaploid wheats. The hypothesis of McFadden and Sears to build up the 6*x* wheats through the spelt form *T. Spelta* seems to be a rather simple and convincing one—and returns to the view of August Schulz and others. Some very grave difficulties arise, however, on historical and geographical grounds.

(1) Hulled forms of hexaploid wheats of the lax type of *T. Spelta* have never been found in Transcaucasia. They are endemic for the upper and middle valley of the Rhine (Switzerland and South-West Germany).

(2) They do not appear in these countries before the Bronze Age, that is to say, later than the Neolithic cereals *T. monococcum*, *dicoccum* and *compactum* (together with a dense six-rowed barley). These two facts led Flaksberger and Schiemann (1939) in accordance with genetic experiments to the hypothesis that *T. Spelta* originated in the Rhine valley at the threshold of the Bronze Age from a cross of the wheats grown there before. It is easy to get hexaploid *Spelta* types from the cross *T. dicoccum* ($4x^-$) × *T. compactum* ($6x^-$).

(3) In order to avoid these difficulties, McFadden and Sears assume the lake dwellers' compact naked wheat ("Binkelweizen") was a *tetraploid* wheat.

(4) This hypothesis again is in disagreement with the fact that compact 6*x* wheats belong to the oldest relics of this small group of cereals comprising "Einkorn, Emmer and Binkelweizen." This group has been cultivated in the lake dwellers' Rhine area since the Neolithic Age and, as far as we know, uninterruptedly (not merely until Roman times as McFadden and Sears presume) although slowly diminishing in the Alps, Thuringia, Sweden, and some other places of Europe (*cf.* Schiemann, 1949).

In spite of the elegant synthesis of a *T. Spelta*-type as an amphidiploid of *T. dicoccoides* × *Ægilops squarrosa* by McFadden and Sears, the problem of the origin of the hexaploid soft wheats is far from being solved. A hypothesis that does not consider the explicitly endemic character of *T. Spelta*—in space and time—cannot claim to be conclusive.* Some of the details are mere working hypotheses themselves. As such they may be accepted and it will be the task of the coming years to continue experimental work and, what is perhaps of equal value, perform a thorough exploration of the areas concerned, especially in the Near East, in order to come to a better knowledge of the distribution and character of the wild and primitive cultivated grasses.

5. NEW MATERIAL FROM PREHISTORIC FINDS

Near East and Egypt.—The international excavations in Egypt and the Near East before the war have brought testimonies of agriculture far back into prehistoric time up to the beginning of settlement. This is especially true for Upper Egypt and the Nile Delta, where we come back to the Neolithic, predynastic period, and in Mesopotamia to pre-Sumerian time (Khafaji). Most valuable are finds of the chalcolithic period between these two border areas, in Syria, Irak, and Anatolia, where till recently little was known of agricultural plants in ancient times. Part of the material, especially that of the British excavations, had already been determined and published by British scientists before the war. But many finds from these areas where German excavators have participated were delivered for determination to E. Werth and to the author. In table 1 of my paper of 1943 I gave a survey of the results of all these contributions which may be summarised in the following way :—

(1) Wherever there are signs of agriculture, barley is present : six- and four-rowed barley, often mixed with two-rowed. In Neolithic Fayum the British have determined naked barley, and so has the author in Assur for the later period. The many-rowed barley is by far the most frequent. In fact, the determination of two-rowed barley rests on the absence of asymmetric grains, and is trustworthy only if much material is available.

(2) Emmer is the only wheat found until now in the area in question from Neolithic time up to the Seventh Century B.C. In Hissarlik (copper bronze period, Troja II) where *T. monococcum* is the chief cereal, the author, in revising the old Berlin material, detected a rather high percentage of *T. dicoccum*. This is of interest in so far as it fills a gap on the ancient east-west route of this cereal.

* In this connection it may be of interest to know that Kihara (1949) who also synthesised *T. Spelta* from the same species, discussing the origin of *T. aestivum* (= *vulgare*), thinks the new theory of McFadden and Sears to be " very unusual " and " the history of the development of the hexaploid soft wheats so far not clear." And " For the ultimate solution we must continue more detailed studies on *Triticum* and *Aegilops* from various standpoints."

(3) A problem arises with the few determinations of hexaploid wheats—resting usually on but one or two single grains, and this more than once with a sign of interrogation (Wittmack 1896 chalcolithic in Bos-öjuk ; in Hissarlik, Troja II ; Harlan *et al.* 1937 in Alishar chalcolithic ; Schiemann Assur). No conclusive proof can be derived from these determinations. Bertsch is right to have pointed out this fact. But it has led him to his theory of the origin of *T. compactum* from *monococcum* × *dicoccum*. And it has led McFadden and Sears to presume the lake dweller wheat to be tetraploid. These theories I have shown not to hold true, though the new archæological results obviously raise again the problem of the origin of the hexaploid soft wheats.

Europe.—Since we intend here to record German contributions of the last decade, only short mention can be made of the very extensive and detailed publications of Hatt (1937), Jessen (1939), and Iversen (1941) for Denmark, which have been followed by those for Great Britain and Ireland (Jessen and Helbaek, 1944), concerning prints of cereals in sherds of vessels.

The German investigations in this internationally planned work, which lay in the hands of the author, have been interrupted and in the end cancelled by the closure and partly by the loss of the German Museum material and cannot yet be started again. German contributions therefore concern either older material or the determination from new excavations in Germany. The most interesting are :—

(1) Cereals from Trebus-Mark, which gave one of the most eastern references for the Neolithic Age. According to Werth (1937) and Schiemann (1940) it contains six- and four-rowed hulled barley and emmer. In connection with the control of some older determinations of single grains as *T. monococcum*, a statistical investigation was made on the dimorphism of *T. dicoccum* (top grains) and *T. monococcum* (one- and two-grained) from the living collection. It proved the determination as Einkorn of single grains of deviating form among emmer to be rather problematic.

(2) A huge find of *T. monococcum* as a staple crop in a late Neolithic settlement in Oldenburg on the Dümmer, determined by Bertsch.

(3) Many new samples of the cereals known from the lake dweller area of South-West Germany, especially from Federsee and Bodensee, determined by K. and F. Bertsch. Their determinations, valuable because of the great number of localities and big specimen (pieces of ears), lack details, especially concerning the quantity in the single finds which makes a comparison with other publications from the same and other localities difficult. These authors give a most complete list * of the finds of *T. monococcum*, *dicoccum* and *compactum ;* yet the text of their paper is not free from errors and the genetic arguments and theoretic consequences, both phylogenetic and historic, are, as we saw earlier, open to criticism.

* There is a complementary list of the Swiss Neuweiler : Nachträge II urgeschichtlicher Pflanzen, 1946.

6. THE ORIGINS OF AGRICULTURE

Since 1937 several new views on an earlier beginning of agriculture arose, founded partly on differential dating of pollen spectra (Jonas and Benrath), partly on ethnological data (Werth). They were followed by a controversy in botanical and archæological papers (Schiemann and Werth, 1940). At present the best-founded view seems to be that agriculture, as indicated by the chief field crops, must have been brought to Europe *in early Neolithic times* by migrating peoples from the east, *i.e.* the areas we considered as places of origin of wheat and barley ; that for Middle Europe, especially for Germany, the Bandkeramik people were the first to appear here as cultivators of these crops, which later, perhaps from tribe to tribe, conquered the whole of the cultivable area of Europe.

7. SUMMARY

New material of cereals from Middle Asia collected by German expeditions in the years 1935-39 gave new results for a better understanding of Vavilov's theory of gene-centres.

(1) Two new finds of wild six-rowed barley, *Hordeum agriocrithon*, have been made in and near Lhasa.

(2) The whole area east of the Hindu Kush has been, and is, cultivating only six-rowed barley.

(3) Transcaucasia is found to be a rich centre of diversity for tetraploid wheats as well as for two- and six-rowed barleys.

(4) Contrary to Vavilov, *Hordeum spontaneum* and *Triticum dicoccoides* are regarded as direct progenitors of cultivated barley and wheat.

(5) Six-rowed barleys are derived from *Hordeum agriocrithon* in East Asia. Their centre of origin is a centre of diversity to-day.

(6) Two-rowed cultivated barleys are of hybrid origin, arising from the contact of six-rowed cultivated barleys with the Transcaucasian types of *Hordeum spontaneum*. This makes clear the similar variability of both and their simultaneous occurrence west of this area.

(7) Hexaploid wheats must have arisen in the common area of *T. dicoccoides* (or *dicoccum*) and *Ægilops squarrosa*, presumably Transcaucasia. Hence their centre of diversity in the Hindu Kush is not their centre of origin.

(8) Abyssinia and the Hindu Kush are secondary centres, where genes developed (from biological causes), mixed and accumulated (from ethnological causes) in a *Stauungscentrum* (accumulation centre).

(9) Consequently Vavilov's theory is valid in some cases but not in others.

(10) McFadden and Sears' theory of the origin of soft hexaploid wheats does not solve the problem, as it does not account for the endemic character of the Spelt in the Rhine valley since its appearance in the Bronze Age.

8. APPENDIX ON NOMENCLATURE

In order to protect the name of *T. vulgare* the Deutsche Botanische Gesellschaft in a session in Berlin in 1941 discussed the possibility of revising the 5th International Botanical Congress' decision rejecting any nomina specifica conservanda. Since 1787 the name of *T. vulgare* for the world's staple food crop has been used in a very wide field of literature, in scientific as well as in practical papers, in Botany and Agriculture, in Taxonomy and Physiology, Genetics and Ecology, in floras and textbooks, in Political Economy and Industry, etc. Difficulties might arise when citing text and tables of former publications for comparison. The necessity of coming to a stabilised nomenclature, compelled me, however, to accept the new rules. In consequence, the following changes have been introduced in the new edition of Engler-Prantl Natürliche Pflanzenfamilien : Gramineæ * and have been used in teaching and publications ever since :—

(1) *T. æstivum*, L., instead of *T. vulgare* Vill. the common hexaploid soft wheat, embracing summer and winter forms. Since Fiori and Paoletti were the first to use the revised name, it ought to be correctly cited : *T. æstivum* L. emend. Fiori et Paoletti ; we propose to put (= *T. vulgare*) in the text in parenthesis, until the new name has become familiar. Experience shows that the younger generation getting accustomed to the new term from the beginning, has no difficulty in accepting it.

(2) In 1946 Schiemann incorporated *T. compactum* Host. in Triticum æstivum under the name of *T. æstivum*—æstivocompactum. It had been regarded as a variety of *T. vulgare* until 1809 when Host gave it the rank of a species. The new change is in agreement with Vavilov's law of homologous series, since the hexaploid compact wheats correspond to parallel groups in the tetraploids, called by Vavilov *durocompactum, turgidocompactum, polonicocompactum.* All these groups comprise compact forms mixed with or to lax types of otherwise equal characters. In 1911 Nilsson-Ehle showed the difference of *T. vulgare* and *T. compactum* to depend on a dominant gene C, which separates abruptly the otherwise continuous character of ear-density.

(3) *T. ægilopoides* Bal. has to be replaced by *T. bœoticum Boissier* emend. Schiemann, the name in Boissier not embracing the whole species. *T. ægilopoides* Forskål (Fl. ægypt. arab. 1775 26) designs *Rottbœllia hirsuta* Vahl (Dansk Arkiv IV 1922 13) and has to be slipped as a later homonym. Flaksberger was not entitled to change the name to *T. spontaneum.* We accept Flaksberger's subspecies' names, subsp. *ægilopoides* and subsp. *Thaoudar*, being the old species names of Link and Reuter.

(4) In 1934 already Nevski replaced *T. persicum* Vav. by *T. carthlicum* Nevski, an older homonym existing in *Ægilops triuncialis* L. as *T. persicum* Aich. et Hemsl.

(5) The same holds good for *T. orientale* Perc. which has an older homonym in *T. orientale* M. Bieb. 1809, now *Eremopyrum orientale* Jaub. et Spach. In 1940 Jacubziner changed the name of this tetraploid naked wheat to *T. turanicum* Jakubz.

With these corrections, except that for *T. turanicum,*† the genus *Triticum* enters the scheme of A. Schulz as shown in Table 1, p. 16, 1946.

(6) Since the wild tetraploid *T. Timopheevi* is shown by Kihara and Lilienfeld (16) to have a genome differing from the other tetraploids, it should prove more correct in future to separate it from the *Dicoccoidea* in a series of its own. This was done in in 1942 by Kihara and Ishikawa.

(7) As far as could be detected, no changes of names had to be made in *Secale* and *Avena* nor in the genus *Hordeum Sectio Cerealia*, the name of *Hordeum vulgare* L. emend. Lam. being valid for cultivated four- and six-rowed barleys, if they are understood—as Schiemann does—as one species parallel to the lax and dense forms of wheat. For revision of the place and name of *Hordeum intermedium* (*cf.* Schiemann 1943, p. 86).

* This paper appeared as a separate book under the title of *Weizen, Roggen, Gerste, Entstehung, Geschichte und Verwertung*, until the manuscript of *Gramineæ II* of Professor Pilger, destroyed by bombing, will be rewritten.

† I thank Mr Hubbard, Kew, for this reference which I could not enter in the scheme of 1946.

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THE PROGRESS OF INBREEDING IN BARLEY

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I. INTRODUCTION

THE genetical effect of inbreeding is to lower, generation by generation, the probability of an individual being heterozygous for any given gene, and correspondingly to raise the probability of it being homozygous for one or other allelomorph. Starting with a single individual, or a group of genetically like individuals (as in the F_1 of a cross between two true breeding strains), the chance of homozygosis for any gene which was initially heterozygous, can be calculated for any generation and any system of mating, on the supposition of mendelian inheritance. With n generations of continuous self-fertilisation, the probability of homozygosis will be $1-(\frac{1}{2})^{n-1}$ as Mendel himself showed. The series for brother-sister mating was given by Jennings (1916), and the consequences of other systems of mating have been worked out, not only for genes showing simple mendelian behaviour, but also where inheritance is complicated by linkage or polysomy (Haldane and Waddington, 1931 ; Bartlett and Haldane, 1934 ; Fisher, 1949).

It is, however, clear that the rise of homozygosis under any mating system will be profoundly affected by any selection, whether natural or artificial, which may be acting within the progenies. If heterozygotes should be favoured the rise of homozygosis will be slowed down, and in the extreme case may even be prevented. Similarly where homozygotes enjoy an advantage, homozygosis will be brought about with greater speed. Calculations based on the assumption of inheritance uncomplicated by selection can lead to expectations applicable only where selection is, in fact, absent ; and the validity of this assumption has become increasingly suspect in many of the species of plants and animals which have been inbred for experimental or for commercial purposes (*e.g.* Düzgünes, 1950).

Where a species normally reproduces by out-breeding it is commonly observed that individuals, which as a result of enforced inbreeding are heterozygous for fewer genes than usual, are also less vigorous than usual. Thus, the more heterozygous individuals will be expected to have an average advantage over their less heterozygous fellows, so that any competition, before seed-shedding or birth as well as in later life, may be expected to slow down the rise of homozygosis. Corresponding differences in fertility must aggravate this situation where the offspring of different individuals are allowed to compete with one another.

With species which normally inbreed, such as barley, differences in vigour and fertility between outbred and inbred individuals are much smaller, though they often exist. Competition will, therefore, be expected to be less decisively in favour of the more heterozygous individuals, so that the rise of homozygosis under inbreeding following crossing will depart less, if at all, from simple expectation.

It may thus be anticipated that the rise of homozygosis under inbreeding will vary from species to species, according to the natural breeding systems. Certainly, calculations based on uncomplicated inheritance cannot adequately represent the situation. Experiments are needed from which can be estimated the speed with which homozygosis is attained.

2. THE ESTIMATION OF PROGRESS UNDER INBREEDING

Where the individual (or individuals) with which the inbreeding programme commenced, was heterozygous for one or more genes of major effect, the progress of inbreeding can be followed by recording the proportions of individuals detectable as homozygous for one or other allelomorph by direct observation or by a simple breeding test. It is obvious that genes whose differences themselves affect the breeding system will be unreliable as indicators, and in any case the estimates obtained by this method will be representative only so far as the effect of the indicator gene on the vigour and fertility of homozygotes and heterozygotes is representative of all the genes that are segregating. Now genes having a drastic effect on the phenotype commonly produce marked disturbances in vigour and fertility. Indicator genes must, therefore, be chosen with care if the results they give are to be accepted as reliable. Major genes suited to the purpose may not, in fact, be available in a species, or at least in the strain or strains whose behaviour under inbreeding it is desired to investigate.

A more generally applicable method for the study of departure of the progress of inbreeding from simple expectation is afforded by the biometrical analysis of continuous variation. Except in long inbred or specially prepared experimental stocks, there will be found heterozygosity and segregation of the members of the polygenic systems mediating continuous variation. Any measure of the rise of homozygosis to which they lead will be an average obtained from a number of genes, and will thus be more generally representative than that afforded by any but a most fortunately chosen single gene.

The biometrical method described by Mather (1949*a* and *b*) can be adapted for the purpose of following the progress of inbreeding. Starting with a single individual, or set of genetically uniform individuals, and assuming the scale upon which the expression of the character is measured to be appropriately chosen as eliminating or at least minimising genic interaction, the variation can be divided into three components, viz. (i) that depending on differences between

comparable homozygotes and denoted by D, (ii) that depending on dominance of the genes and denoted by H, and (iii) that depending on non-heritable agencies and denoted by E. The different second degree statistics (variances and co-variances) which can be calculated from the various generations in a suitably designed experiment, can be expressed in terms of D, H and E; and their observed relations can be compared with those expected on the supposition of uncomplicated inheritance under the mating system in force.

Thus the variance of an F_2 can be expressed as $\frac{1}{2}D+\frac{1}{4}H+E_1$. The F_3 generation, raised by the self-fertilisation which occurs naturally in barley, yields three second degree statistics, the mean variance of families which is $\frac{1}{4}D+\frac{1}{8}H+E_1$, the variance of family means which is $\frac{1}{2}D+\frac{1}{16}H+E_2$, and the co-variance of F_3 family mean with F_2 parental value which is $\frac{1}{2}D+\frac{1}{8}H$. It will be observed that the E component of the variance of family means is denoted by E_2 to distinguish it from E_1, occurring in the variance of F_2 and mean variance of F_3's. This is necessary because the non-heritable variation of a mean will differ from, and generally be less than, that of single individuals. The co-variance will, of course, have no non-heritable component in a properly designed experiment.

The F_4 generation can yield five second degree statistics, since we can recognise not merely different families as the offspring of single F_3 individuals, but also different groups of families as the descendants of single F_2 individuals. The complexity of classification and the multiplicity of statistics increases correspondingly as we move on to F_5 and later generations.

The barley experiment to be described was conducted (see Section 3) in such a way that only five statistics could usefully be found in each generation even after F_4, viz. :—

(1) The mean variance of families.
(2) The variance of line means, which is the equivalent of the variance of group means in F_4.
(3) The variance of family means within lines (and averaged over lines), which is composite after F_4.
(4) The co-variance of parental and offspring line means, which is the counterpart of (2), and
(5) The co-variance of parent value and mean of offspring within lines (and averaged over lines) which is the counterpart of (3).

The expressions for these statistics are given in table 1. In F_3 of course, statistics (3) and (5) are not computable.

The heritable components of the variances can be added in each generation to give an expression for the total free variation. It will be observed that when this is done, the coefficient of D follows Mendel's series for the chance of an individual of that generation being homozygous for one or other allelomorph of a gene heterozygous in the F_1.

TABLE 1

The components of variation under inbreeding by self-fertilisation (see text for explanation of statistics (i)-(v))

Statistic / Generation	(i)	(ii)	(iii)	(iv)	(v)	Sum of heritable components of (i), (ii) and (iii)
F_2	$\frac{1}{2}D+\frac{1}{4}H+E_1$	...	...	...	...	$\frac{1}{2}D+\frac{1}{4}H$
F_3	$\frac{1}{4}D+\frac{1}{8}H+E_1$	$\frac{1}{2}D+\frac{1}{16}H+E_2$	...	$\frac{1}{2}D+\frac{1}{8}H$	...	$\frac{3}{4}D+\frac{3}{16}H$
F_4	$\frac{1}{8}D+\frac{1}{16}H+E_1$	$\frac{1}{2}D+\frac{1}{64}H+\frac{E_2}{n}$	$\frac{1}{4}D+\frac{1}{32}H+E_2$	$\frac{1}{2}D+\frac{1}{32}H$	$\frac{1}{4}D+\frac{1}{16}H$	$\frac{7}{8}D+\frac{7}{64}H$
F_5	$\frac{1}{16}D+\frac{1}{32}H+E_1$	$\frac{1}{2}D+\frac{1}{256}H+\frac{E_2}{n}$	$\frac{3}{8}D+\frac{3}{128}H+E_2$	$\frac{1}{2}D+\frac{1}{128}H$	$\frac{3}{8}D+\frac{3}{64}H$	$\frac{15}{16}D+\frac{15}{256}H$
F_6	$\frac{1}{32}D+\frac{1}{64}H+E_1$	$\frac{1}{2}D+\frac{1}{1024}H+\frac{E_2}{n}$	$\frac{7}{16}D+\frac{7}{512}H+E_2$	$\frac{1}{2}D+\frac{1}{512}H$	$\frac{7}{16}D+\frac{7}{256}H$	$\frac{31}{32}D+\frac{31}{1024}H$
F_7	$\frac{1}{64}D+\frac{1}{128}H+E_1$	$\frac{1}{2}D+\frac{1}{4096}H+\frac{E_2}{n}$	$\frac{15}{32}D+\frac{15}{2048}H+E_2$	$\frac{1}{2}D+\frac{1}{2048}H$	$\frac{15}{32}D+\frac{15}{1024}H$	$\frac{63}{64}D+\frac{63}{4096}H$
F_8	$\frac{1}{128}D+\frac{1}{256}H+E_1$	$\frac{1}{2}D+\frac{1}{16384}H+\frac{E_2}{n}$	$\frac{31}{64}D+\frac{31}{8192}H+E_2$	$\frac{1}{2}D+\frac{1}{8192}H$	$\frac{31}{64}D+\frac{31}{4096}H$	$\frac{127}{128}D+\frac{127}{16384}H$
F_9	$\frac{1}{256}D+\frac{1}{512}H+E_1$	$\frac{1}{2}D+\frac{1}{65536}H+\frac{E_2}{n}$	$\frac{63}{128}D+\frac{63}{32768}H+E_2$	$\frac{1}{2}D+\frac{1}{32768}H$	$\frac{63}{128}D+\frac{63}{16384}H$	$\frac{255}{256}D+\frac{255}{65536}H$

The coefficient of D in generation F_n is $1-(\frac{1}{2})^{n-1}$, while the coefficient of H approaches $(\frac{1}{2})^{n-1}$ as n becomes large. It is, however, always less than $(\frac{1}{2})^{n-1}$ by an amount $(\frac{1}{2})^{2(n-1)}$, thereby suggesting that the measure of total free heritable variability is omitting an item of size $(\frac{1}{2})^{2(n-1)}$. This item is, in fact, represented by the departure of the mean measurement of the whole generation from the mid-parent value, *i.e.* from the mean of the measurements of the two true-breeding parental strains by crossing which the F_1 was produced or could have been produced. In Mather's notation the mean of F_1 departs from the mid-parent by $S(h)$ where h is the departure caused by heterozygosity for a given gene from the mean of the two corresponding homozygotes. The departure of the F_2 mean from the mid-parent is $\frac{1}{2}S(h)$, that of F_3 is $\frac{1}{4}S(h)$ and that of F_n is $(\frac{1}{2})^{n-1}S(h)$. Now H is compounded of quadratic terms in h. Squaring the coefficient of $S(h)$ in a corresponding way gives, for F_n, $(\frac{1}{2})^{2(n-1)}$ which is the item required to complete the balance sheet of variability. When it is included, the coefficients of D, H and $S(h)$ sum to 1. The free heritable variability is accounted for completely by reference to the three alternative states in which it can exist and which we regard as contributing to D, H and $S(h)$ respectively (Mather, 1949*b*).

Where the inbreeding programme commences with a single individual or a uniform F_1, produced by crossing two true-breeding strains, the total heritable variability as measured in this way will be constant over the generations in the absence of selection, provided that the genes are unlinked. The effect of linkage is to change the values of D and H from generation to generation. With preponderantly coupling linkage, D declines in value as the generations proceed. With reinforcing dominance, H declines in the same way. Preponderantly repulsion linkage and opposing dominance lead to D and H respectively increasing in value with the generations (Mather, 1949*a*).

The distribution of variability, though not of necessity its total, will also be affected by selection, the precise effect depending on the nature of the selection itself. Where one allelomorph of the gene is favoured at the expense of the other, the values of D and H change (Mather, 1949*a*) ; but where the effect is that of favouring heterozygosity as opposed to homozygosity, or *vice versa*, the consequence will be seen in the coefficients of D and H. With heterozygosity favoured, the coefficients of D will be lower than those expected, and those of H higher by amounts which increase progressively with the generations. With homozygosity favoured, the departures will go in the opposite direction. In the extreme case where only the individuals heterozygous for all genes contribute to the next generation, the coefficients of D and H will remain at $\frac{1}{2}$ and $\frac{1}{4}$ respectively instead of changing with the series $1-(\frac{1}{2})^{n-1}$ and $\frac{1}{2}^{n-1}(1-\frac{1}{2}^{n-1})$. At the other extreme where only the various homozygotes breed, the coefficient of D will be 1 and that of H will be 0 in every generation after F_2. These extremes have,

however, little more than academic interest, for we can expect to meet them rarely, if ever.

Thus, in theory, linkage and selection have separable effects, on the values and coefficients of *D* and *H* respectively (figs. 1 and 2). In practice they will be more difficult to distinguish. When we analyse data we shall find that the effects of both linkage and selection will appear as changes from generation to generation in the values of *D* and *H* if these are permitted to vary, or in their coefficients if they are not. Doubtless some separation of their effects is possible by suitable analysis, but this has yet to be attempted. For the present it is sufficient to note that where homozygotes are favoured the result will resemble (at least superficially) that of coupling linkage, and where heterozygotes are favoured the result will resemble that of repulsion linkage, figs. 3 and 4.

One point remains to be noted before we turn to the experimental results obtained with barley. This cereal naturally self-pollinates, and our discussion of the use of the biometrical method in the study of inbreeding has, therefore, been based on the assumption of this breeding system. The consequences of other breeding systems, leading for example to brother-sister or cousin mating, can, however, be represented by reference to *D* and *H* in the same way. It is the properties of our experimental material that leads us to discuss inbreeding in terms of self-pollination : the method of analysis is in essence a general one.

3. THE EXPERIMENT

The experiment began as far back as 1938, with a cross between the two barley varieties, Spratt and Irish Goldthorpe. The cross was made for the production of large F_2 and F_3 families for the biometric analysis of *D* and *H* published elsewhere (Mather and Philip—see Mather, 1949*a*). All the plants used for that analysis were grown in 1941. In that year some of the ears on five F_2 (*A*) plants and two F_3 (*B*) plants were bagged and some ears were left unbagged. These seven plants were the source of all the later generations dealt with in this paper, each giving rise to one of the seven lines. Twenty seeds from the bagged ears and twenty from the unbagged ears were sown in a randomised block in 1942, producing 14 families. The progenies of bagged and unbagged ears formed duplicate series designated *b* and *u*.

Ears were bagged on one or two plants in each family in 1942. These plants produced 20 *b* and 20 *u* families in 1943. From 1942 to 1945 inclusive all the families were grown together in a single randomised block. Each 1943 family had *b* and *u* seed saved from one or two plants in such a way that every 1942 family was represented in 1944 by 8 families (4 *u* and 4*b*), making 112 families in all, each of 10 plants. The method of producing two families from one plant in each family of the previous generation had doubled the number

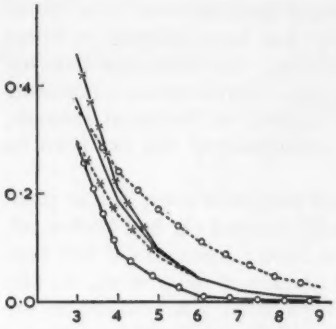

FIG. 1.—Mean Variance.

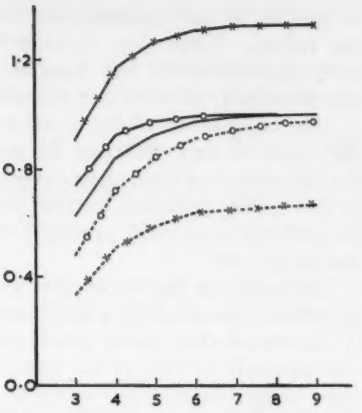

FIG. 2.—Covariance of Parent and Progeny.

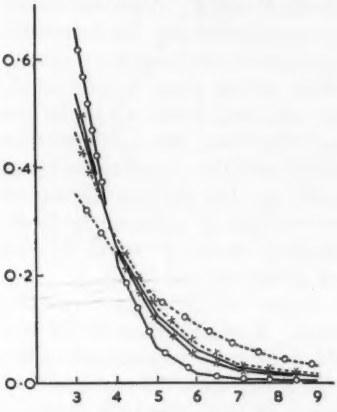

FIG. 3.—Mean Variance.

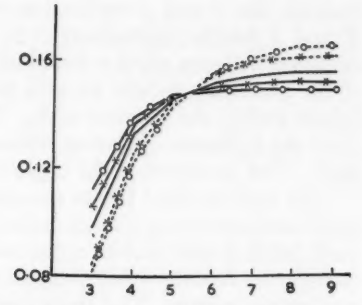

FIG. 4.—Covariance of Parent and Progeny.

General legend to figs. 1-4

The effects of linkage and selection on Mean Variance and Parent-offspring Covariance from F_3 to F_9.

———————— Simple inheritance.
×——×——× Linkage in coupling.
× - - - × - - - × Linkage in repulsion.
o——— o ———o Selection favouring homozygotes.
o - - - o - - - o Selection favouring heterozygotes.

There are assumed to be two genes $A-a$ and $B-b$ of equal effect with a linkage value (when linked) of 0·25 and a selection ratio (when effective) of 2 : 1. In figs. 1 and 2, $d_a = d_b = h_a = h_b = \sqrt{\frac{1}{2}}$, and the general levels of Mean Variance and Covariance over the generations vary with the linkage and selection. In practice, however, the general levels of these statistics are given and it is the changes from generation to generation with which we are concerned. In figs. 3 and 4, therefore, whilst $d_a = d_b = h_a = h_b$, their values vary in such a way that the sum of the statistics from F_3 to F_9 is constant at unity.

In figs. 3 and 4 the linkage curves deviate from simple inheritance less than the selection curves. With more linked genes, however, the deviation would be increased.

of families in each generation. In 1944 the multiplication of lines was halted. From then on only half the families of one generation were represented by two families in the next, the other half being unrepresented, so stabilising the number of families.

The comparison of the *u* and *b* series had been designed to detect the origin of new variation by outcrossing. No difference between the two series had been observed up to 1944. Furthermore, outcrossing in this barley material seemed most unlikely on botanical grounds, for anthesis invariably preceded the emergence of the ear from its sheathing leaf.

Insistence on the recovery of *u* and *b* seed from every parent plant sometimes meant losing a line because the bagged ear had broken off. It also meant that parent plants had to have a minimum of two ears. Consequently in view of the apparent absence of outcrossing, no ears were bagged from 1945 onwards. Replication was maintained by dividing the seed from each parent plant into two equal lots *x* and *y* which were sown in two randomised blocks *X* and *Y*. A further break in the uniformity of treatment in the experiment was inadvertently made in 1946. In this year alone the practice of obtaining the replicate families (*u* and *b* or *x* and *y*) from a single parent plant was dropped. Instead, the *x* and *y* replications were obtained from plants in the *X* and *Y* blocks respectively. In 1947 therefore, the non-heritable variation between *x* and *y* was confounded with the sampling variance of the parents. As will be seen from fig. 3A this did not appear to inflate unduly the estimate of E_2. Nor would it be expected to do so, since the heritable variation within families should be small by this time. The pedigrees of the experiment is summarised in fig. 5.

The experimental blocks consisted of rows one foot apart with the seeds hand-sown singly at six inches apart. A modification of this was made in 1947 and 1948 in order to reduce the error engendered in the statistics by families containing markedly fewer than the maximum of ten mature plants. The seeds were sown in pairs one inch apart at right angles to the rows. When germination had been completed the seedlings were singled. This improved the stand at harvest though it was still incomplete.

Since the analysis of the experiments is based on a comparison between generations it is important that each family in one generation be represented equally in the next generation. Otherwise the comparison between generations is of doubtful value. For this reason, as has been stated above, each 1941 parent plant was represented by 16 families in 1944. After 1944, however, a number of family sequencies died out. In order to maintain the balance double weight was given to the most closely related family, in calculating the statistics. If the families were lost at random this would be the best treatment. The analysis of the data which follows gives, however, good cause to suspect the action of selective loss of families. Statistics obtained by the system of weighting adopted would tend to mask the action of

selection. They have therefore been recalculated, giving equal weight to all surviving families at each generation.

The previous analysis of barley data (*loc. cit.*) was carried out on a discriminant function involving ear length, ear breadth and the length

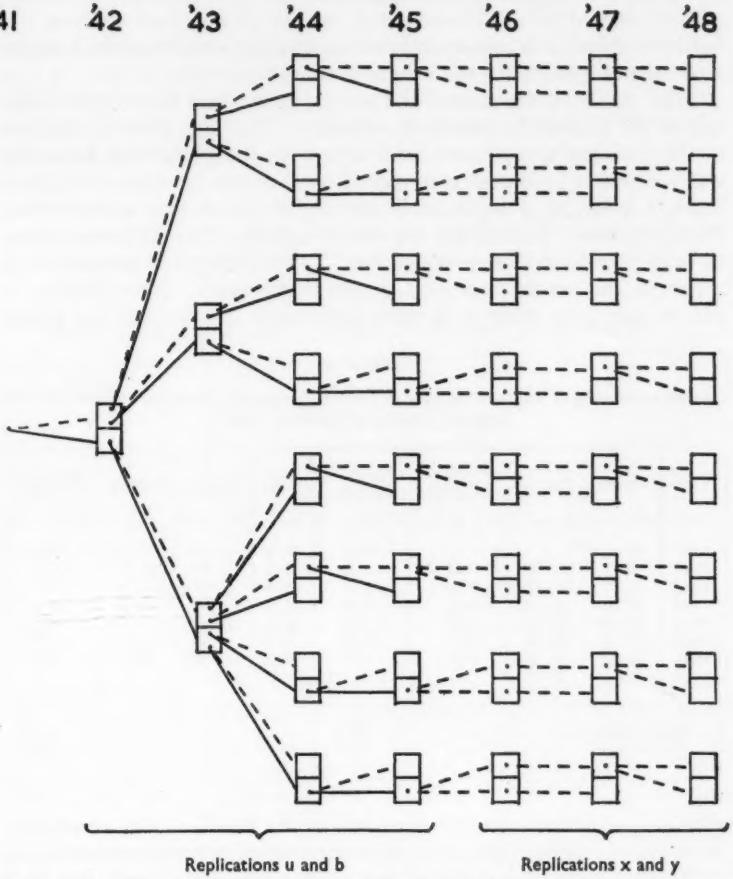

FIG. 5.—The pedigree of all the families within one line, *i.e.* descended from a single 1941 plant. Each square represents a family ; each dot within a square, a parent plant. Entire lines, descent via a bagged ear ; broken lines, by an unbagged ear. The paired squares represent replicates from which the duplicate statistics were obtained. For further details see text.

of the middle twelve internodes (the length taken up by seven nodes on one side of an ear). Since the greatest weight was given to internode length it has been thought sufficient to take the internode length alone in the following analysis, thus saving a great deal of labour.

Fourteen internodes were measured from the first. When it was discovered that the earlier discriminant had been based on twelve internodes, it was thought not worth while altering the metric. One ear taken at random was used to represent each plant. The measurements were all expressed in millimetres.

In so far as we are concerned to see the effects of selection on the barley statistics, it is appropriate to consider the opportunities it might have had of influencing the results of the experiment.

The choice of the parent of the next generation from each family was as far as possible genetically random. The fifth plant in the row of ten, or what would have been fifth if all ten plants had matured, was taken if it produced a minimum of two ears at time of bagging (for u, b series), or a minimum of one mature ear at time of harvesting (for x, y series). Failing this the nearest suitable plant to it was taken. In only a small minority of families was the fifth plant unsuitable as a parent (no record was kept of the proportion). Nevertheless, as can be seen from table 2, in every generation the mean of the plants

TABLE 2

Comparison of mean of parents with mean of families from which they came and mean of their progeny (families of following year)

Year	Parent	Family	Parent—Family	Parent—Progeny	Line	Parent	Family	Parent—Family
1941	36·43	...	...	*0·76*	A 4	25·38	24·98	*0·40*
1942	36·11	35·67	*0·44*	*0·44*	A 5	31·66	30·51	*1·16*
1943	35·87	35·67	*0·20*	*1·38*	A 8	47·28	45·59	*1·69*
1944	34·85	34·49	*0·36*	*1·34*	A 9	32·66	32·25	*0·41*
1945	35·99	33·51	*2·48*	*−1·57*	A10	46·56	44·41	*2·15*
1946	38·26	37·56	*0·70*	*−0·54*	B 1	30·32	31·20	*−0·88*
1947	39·57	38·80	*0·77*	*1·50*	B 5	43·56	42·71	*0·85*
1948	...	38·07	...	...	...	...	...	...
Mean Difference .			*0·825*	*0·473*				*0·826*
Probability . .			*0·05*	*0·3*				*0·05*

used as parents was greater than that of the families from which they were drawn. That this excess was not confined to certain constitutions is shown by the same comparison analysed by lines (each line is, it will be recalled, descended from a single 1941 plant) instead of generations. In both cases the excess of the parental means just reaches the 5 per cent. level of significance. In the comparisons according to lines there is evidence that the excess increases with the mean.

That the excess of the parent plants is partly non-heritable is shown in the parent-progeny comparison (the difference between parent mean of one year and family of the next). Here the error variance is naturally higher, since the comparison involves the different

environments of the two years. The mean difference is still positive, however, but it is smaller and has no statistical significance.

Some measure of the scope for selection is given by the variation in fertility and survival rate. If all families gave a complete stand of mature plants there would be no opportunity for selection except at the gametic level. This was far from the case. Table 3 shows the variation in fertility expressed as number of mature plants obtained out of a maximum possible of 10. In the years up to 1947 the deficiency stems from four causes: failure of the parent to give sufficient seeds for complete sowing; poor germination of the seed; post-seedling death of the plant and failure of the plant to produce ears. In 1947 and 1948, as will be remembered, two seeds were sown for every one plant required. This reduces the effect of poor germination but the other factors are unaffected. The variation between lines in fertility as shown in table 3 is highly significant

TABLE 3

Fertility as measured by number of plants surviving out of the ten required

Line \ Year	1942	1943	1944	1945	1946	1947	1948	Line Mean
A 4	10·00	8·25	4·38	5·88	5·13	7·57	5·13	*6·62*
A 5	9·75	9·13	7·36	6·34	6·88	8·38	7·38	*7·81*
A 8	10·00	9·63	8·00	8·00	7·93	9·43	8·43	*8·77*
A 9	6·50	9·38	6·75	6·94	4·72	7·23	8·43	*7·14*
A10	9·00	9·50	7·19	5·08	6·30	8·63	7·63	*7·62*
B 1	10·00	9·13	7·13	7·00	6·88	8·75	6·63	*7·93*
B 5	9·00	9·50	6·88	8·08	5·93	8·50	7·57	*7·92*
Yearly Mean	**9·18**	**9·22**	**6·81**	**6·76**	**6·18**	**8·36**	**7·31**	...

(probability ·001). The variation from year to year is even greater. The variation between lines is mainly due to the high fertility of A8 (high mean internode length, 40-50 mm.) and low fertility of A4 (lowest length, around 25 mm.) and A9 (moderately low length, around 30 mm.). This is further evidence of selection against low values.

The year to year variation in fertility is mainly due to a steady fall from 1942 to 1946. With the new sowing technique it rose markedly in 1947 and 1948. Poor seed germination is thus an important factor in lowering fertility, though not the only one. The steady fall in the earlier generations is evidently connected with loss of vigour as a result of inbreeding. In this connection it is worth noting that evidence of heterosis in respect of the ear characters was obtained by Mather and Philip (1949*a*). The results could, however, be explained without reference to hybrid vigour. The two parental forms were internally very well balanced as a result of selection in the homozygous state. The balance might remain in the F_1 which

would contain the two balanced systems side by side. On inbreeding, however, various homozygous combinations of the two parental gene systems would be produced and it would be surprising if these novel combinations were all well balanced. Such inbreeding depression is of a different order of magnitude from that obtained in outbreeding organisms like maize and *Drosophila*. The marked depression in maize, greater than that of the barley though it is, is even then obtained in material continuously subject to upward selection by man.

It may be asked why in F_3 and F_4 there is an almost complete stand, though these generations should show 0·75 and 0·875 homozygosity respectively. It must be borne in mind that we are here concerned not with vigour as such but ability to produce a complete stand of mature plants. It might well be that considerable reduction in vigour can occur before the threshold is passed which reduces the stand. Furthermore, two of the most important factors, number of seeds available for sowing and the germination rate, are largely maternally determined, that is, affected by the previous generation with twice the heterozygosity.

In only one year (1945) was a record kept of the number of grains harvested from the two ears of each parent plant. Only for that year, therefore, is it possible to obtain an idea of the relative importance of infertility of the parent and failure of seeds to produce mature plants. In 1945, of 42 parent plants, 13 produced less than the required number of seeds (twenty). As a result of the infertility of these parents the mean number of seeds sown per parent plant was 17·26. The mean number of plants harvested per parent was only 12·50. In this year the period from seed to mature plant was a more important field for the operation of natural selection than the fertility of the parents.

4. ANALYSIS OF THE RESULTS

(i) *Means*

The grand mean of the experiment shows little evidence of any general trend during the course of the experiments, though the means are higher during the years 1946-48 than earlier. This could, of course, be due to seasonal effects.

When, however, the data are broken down so as to show the behaviour of each of the seven lines, complications become apparent (fig. 6). There is a clear tendency for the lines to diverge from one another, while keeping the same grand mean, at least up to 1945. The overall change in mean from 1946 onwards makes interpretation of this later part of the experiment more difficult.

The interpretation to be placed on this divergency of the lines is not obvious. It will be seen from the figure that various families died out in the early years, but these show little evidence of being distributed in such a way as to cause the effect observed. It would seem that we must rather attribute the effect to differential survival within families ;

and indeed our discussion of fertility has already shown ample scope for divergence to arise in this way.

The remarkable feature of the results is that such selection should give rise to divergence. This must mean that genotypes of intermediate effect were at a disadvantage, in spite of the fact that the two parents were actually of intermediate internode length. The parents were,

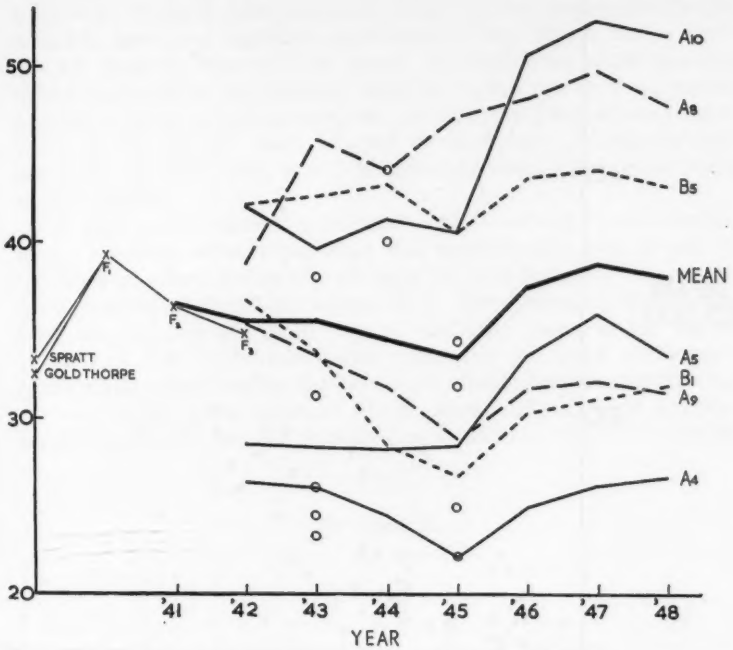

FIG. 6.—The generation to generation variation in the Grand Mean, ear measurement (thick black line), and in the 7 line means making up the Grand Mean. The thin line with the ×'s represents Mather and Philip's means for the corresponding generations which were, in fact, all grown in the same season (1941). The circles show the means of families which failed to produce any offspring.

however, intermediate because of the balancing action of two groups of linked genes opposed in their effects in each parent (Mather, 1949*a*). In the offspring of their cross further genotypes of intermediate effect would be expected from recombination in these linked groups, and it may well be that these genetically new intermediates are the ones against which selection has been acting, so bringing about the divergence. In fact, such an assumption implies that the genotypes produced by recombination within the linkage groups are at a disadvantage, as compared with those others of more extreme effect on the internode length arising by re-assortment of the unbroken

groups. This is in keeping with observations on the course of selection in *Drosophila* (Mather and Harrison, 1949).

It is of interest in this connection that the present observations afford some confirmation of Mather, and Philip's view that the parents differed in two groups of linked genes opposed in their effects on internode length.

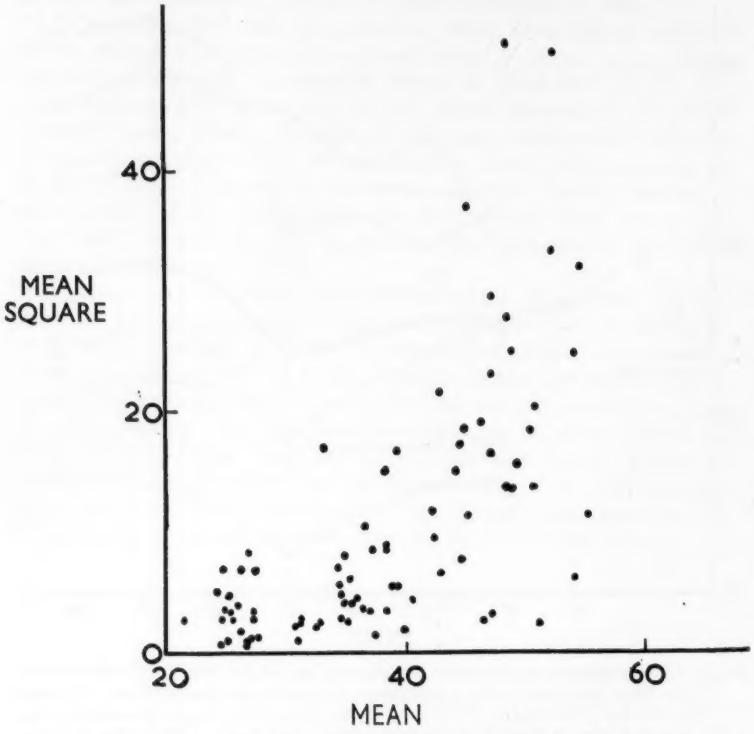

FIG. 7.—Comparison of Mean Square and Mean for all families grown in 1948 (F_9). If the scaling were perfect there would be no relation between the two statistics, as there clearly is.

Such selection against intermediate phenotypes is not one of particular genes as such, but one of particular combinations of these genes. It need not, therefore, upset the individual gene frequencies, nor even the relative frequencies of homozygosis of the individual gene and indeed, the behaviour of the grand mean shows little evidence of such upset. It will, however, affect certain of the statistics, as we shall see later. No change of scale could compensate for its effects.

A further feature of the material which will have its effect on

certain of the statistics could perhaps be overcome by rescaling. In 1948, when the lines would be expected to have attained a high level of homozygosis, the means and variances of the various families were plotted against one another. The variances should be almost entirely non-heritable and, on a fully adequate scale, should be equal, or at least uncorrelated with the means, since they will reflect vagaries of the environment to which all families have an equal chance of being subjected. As fig. 7 shows, however, there is a clear positive correlation of family mean and variance, the variance rising, in fact, rather more quickly than the square of the mean. A logarithmic transformation might well remove this metrical bias, but it has not been attempted because of the great labour involved. In the case of the mean variance which is the statistic most affected by such bias, an approximate correction has been used, as will be explained when attention is turned to that statistic.

A final point remains to be made before turning to the analysis of the statistics. The experiment was conducted in such a way as to afford no direct means of estimating the non-heritable variation to which individual plants were subject (E_1). The comparison of progenies from bagged and unbagged ears of the same plant, in the earlier years, and the comparison (after correcting for block differences) of the x and y duplicates in the later years affords a means of estimating the non-heritable variation of family means (E_2). These statistics are set out line by line and year by year in table 4. There is a decline

TABLE 4

Variation in E_2

Year / Line	1942	1943	1944	1945	1946	1947	1948	Line Mean
A 4	...	0·69	1·40	0·96	0·43	2·29	0·73	*1·08*
A 5	...	1·60	2·46	2·31	3·68	0·50	1·65	*2·03*
A 8	...	8·53	4·49	7·15	4·73	1·70	0·80	*4·56*
A 9	...	1·73	1·58	2·01	3·30	8·16	1·39	*3·03*
A10	...	5·01	1·54	4·65	6·15	3·01	0·58	*3·49*
B 1	...	0·62	1·76	1·07	0·45	2·48	2·28	*1·44*
B 5	...	2·82	10·16	1·95	3·74	0·85	3·45	*3·83*
Yearly Mean	4·61	3·00	3·34	2·87	3·21	2·71	1·56	...

in 1947 and 1948, as would indeed be expected from the precautions taken to ensure a better stand and so more plants per family in these later years. There is also a correlation of line E_2 with the line internode length, as would be expected from the relation of mean and variance already noted. These differences are, however, small when compared with the changes observed in the statistics as a result of the inbreeding. We may thus take it that E_2, and by inference E_1,

are sufficiently near to being constant to cause us little trouble in analysis and interpretation of the experimental results.

(ii) *Second degree statistics*

Before turning to the analysis of the variances and covariances, two points require mention. The first concerns the group of lines designated by *B*. This originated from a single F_3 family, *i.e.* from one F_2 plant. For the purpose of analysis, therefore, we can regard this F_2 plant as corresponding to an F_1, the F_3 family to an F_2 and all the later generations to a generation one earlier than their formal place in the complete experiment would indicate. This course has been adopted in the analysis that follows. It may be noted in this connection that the series *B*, viewed in this way, originates from an F_1 plant which may have less, but cannot have more, genes heterozygous (and hence variability) than the original F_1 which gave rise both to it and, more directly, to the series of lines designated by *A*. Furthermore, any linkage the genes may show will not necessarily be the same in *B* as in *A*.

Secondly, all the statistics (means, variances and covariances) in both series *A* and *B*, were observed in duplicate. This was made possible by growing duplicate families from each parent (except in 1947). In such a system the differences between duplicates reflect sampling variation within families but not between parents. Since this latter sampling variation must have its effect on the changes of the statistics from generation to generation, and such sampling effects in parents must have been large especially in the early generations where few parents were used, the differences between duplicate observations will generally be too small to provide (except in 1947), an estimate of the error with which the changes among the statistics can fairly be compared. No use has, therefore, been made of these differences, and the means of the duplicate observations have been utilised throughout. The comparison in behaviour of *A* and *B* affords a general idea of the reliability of the results.

The F_6 generation contained a number of families in the *A* series which produced no F_7 progeny, the absence of which could not be made up by weighting as there were no close relatives to be weighted. For this reason two values are given for each statistic in F_6 of series *A*, the one being strictly comparable with its counterparts from earlier generations and the other with those from later ones. The data seemed, however, not to warrant the extra labour of making these comparisons separately. An average was therefore taken of the two values of each statistic in F_6 and these averages used in the consideration of the changes in *A*.

In the absence of selection the variance of F_2 is expected to be $\frac{1}{2}D+\frac{1}{4}H+E_1$, where E_1 represents the non-heritable component of variation between individual plants. With simple inheritance,

uncomplicated by linkage or selection, the heritable portion of the mean variance of families is halved in every generation, the mean variance of F_3 ($\overline{VF}_3$) being $\frac{1}{4}D+\frac{1}{8}H+E_1$ and so on. The E_1 item, of course, appears every time. Since no control families were used and the different generations were grown in different years and on different

TABLE 5

Mean variance (weighted data)

Generation (F)	Series *A*				Series *B*			
	Observed			Expected	Observed			Expected
	Duplicates		Mean		Duplicates		Mean	
3	20·11	29·48	24·80	*22·79*	29·34	24·71	27·03	*24·09*
4	10·70	11·60	11·15	*15·19*	8·41	7·67	8·04	*15·23*
5	9·53	14·37	11·95	*11·39*	11·06	17·77	14·41	*10·80*
6 inclusive	7·55	8·82	8·48	*9·49*	5·83	4·96	5·39	*8·58*
6 exclusive	8·12	9·40						
7	6·91	6·85	6·88	*8·54*	8·31	10·21	9·26	*7·48*
8	8·02	11·79	9·90	*8·07*	7·67	7·15	7·41	*6·93*
9	10·06	10·19	10·13	*7·93*	7·76	8·63	8·20	*6·65*

plots of ground, there can be no certainty that E_1 did not itself vary from generation to generation. It will be assumed, however, that E_1 was in fact constant. There is, as we have seen, some support to be obtained from the data for this assumption. It will be observed that H declines proportionately with D over the generations. There is therefore no need to discuss the effect of dominance as a separate consideration ; we require only estimates of $D+\frac{1}{2}H$ and of E_1. Using the method of least squares on the weighted data from F_3–F_9, these are $D+\frac{1}{2}H = 60{\cdot}80$ and $E_1 = 7{\cdot}59$ for A, and $D+\frac{1}{2}H = 70{\cdot}86$ and $E_1 = 6{\cdot}37$ for B—estimates which are in satisfactory agreement with one another and with the evidence of Mather and Philip (Mather, 1949*a*). These estimates can be used to construct expectations for the mean variances of the various generations in table 5.

The comparison of observed and expected values is perhaps clearer from fig. 8 than from the table. In A the tendency seems to be for the observed value to exceed the expected at first and to fall below it in the middle generations, again approaching and even surpassing expectation at the end. The comparison of expectation and observation in B is less satisfactory since the fluctuations of observation are greater, but it certainly cannot be taken as contradicting the conclusions to be drawn from A.

In view of the relation between mean and variance, to which attention has already been drawn, the slight rise in the grand mean

at the end of the experiment could lead of itself to some rise in mean variance. An adjustment was made for this effect in considering the unweighted data. *A* and *B* were pooled for this purpose, as the analysis of the mean squares of the weighted data has given no reliable indication of disagreement between these groups.

The mean variance for each line in each year was divided by the square of the corresponding mean before averaging over lines. This should go a long way towards removing the metrical bias to be expected in the mean variances. The analysis then proceeded along the same lines as with the weighted, but unadjusted, data described above. The adjustment for metrical bias had the effect of lessening the departure of observation from expectation ; but even so a departure of the kind found earlier was still in evidence. The value found for $D+\frac{1}{2}H$ by taking the adjusted figure obtained from this analysis and multiplying by the square of the mean of all family means was

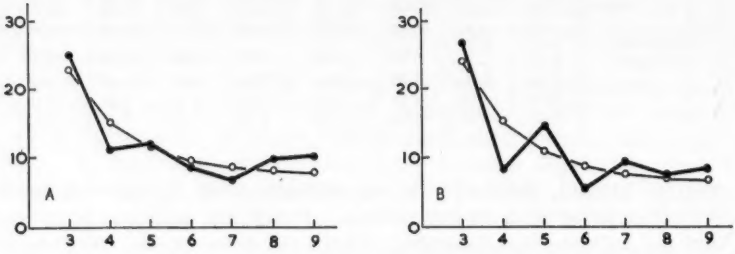

FIG. 8.—Mean Variance (weighted data). Heavy line with black circles, observed values ; thin line with open circles, expectation.

60·44. This agrees quite well with the figures from the earlier analysis. E_1 was similarly found as 6·24.

Whether a fully adequate adjustment of the scale would have removed all the departure is impossible to say. One fact suggests that it would not. The analyses described were made on the data from generations F_3—F_9 : the variance of F_2 was not included in either of the above analyses because of the small number of plants available. The value observed for variance of F_2 was 58·29, as compared with 25·44 for mean variance of F_3. In so far as the F_2 figure is trustworthy it would indicate a too rapid fall between F_2 and F_3. This departure is, of course, of the kind we have already discovered, and in the case of the F_2—F_3 fall it is unlikely to be due to metrical bias, for the difference between the means of the two generations is very small.

It is worthy of note that taking E_1 as 7·0, *i.e.* an approximate value lying between the estimates obtained from the weighted and unweighted analyses, $\frac{1}{2}D+\frac{1}{4}H$ of F_2 is 51·29 while $\frac{1}{4}D$ and $\frac{1}{8}H$ from F_3 is 18·44 (unweighted figures). Thus there has been a fall of

$\frac{(51{\cdot}29-36{\cdot}88)100}{51{\cdot}29} = 26$ per cent. in the value of $D+\frac{1}{2}H$ from F_2 to F_3. This is in close agreement with the fall ratio of 27 per cent. found by Mather and Philip for the compound ear character that they used. We are therefore encouraged to believe that the departure of the series of mean variances from the simple mendelian expectation is due to something more deep-seated than mere metrical bias. In so far as the departure is reliable it would indicate coupling linkage or selection favouring homozygotes. Mather and Philip ascribed the corresponding change that they observed to coupling linkage.

The covariance of individual parent value with mean of offspring contains no non-heritable (E) component. Since no separation was made into lines in this analysis the expectation of the covariance for each generation is the sum of the two items in columns (iv) and (v) of table 1. It will be seen that this expectation contains D and H components whose relative proportions change with generations. The progress of the changes in covariance is thus not independent of the degree of dominance. Two analyses have therefore been undertaken of the covariances weighted to compensate for the loss of lines by infertility. One analysis assumes the absence or balance of dominance for all genes ($H = 0$), and the other assumes an average of complete, but not excessive, dominance ($H = D$). Assuming $H = 0$, D is estimated, from the sum of the covariances of all seven generations tested, as 90·97 in series A and 75·13 in series B. With dominance

TABLE 6

Covariance of parent (F_{n-1}) *and progeny* (F_n) (*weighted data*)

Generation (F)	Series *A*					Series *B*				
	Observed			Expected		Observed			Expected	
	Duplicates		Mean	$H = 0$	$H = D$	Duplicates		Mean	$H = 0$	$H = D$
3	51·34	47·89	**49·62**	*45·49*	*53·90*	23·10	14·17	**18·64**	*37·56*	*44·51*
4	45·59	44·73	**45·16**	*68·23*	*72·77*	66·00	68·34	**67·17**	*56·35*	*60·09*
5	68·47	68·19	**68·33**	*79·60*	*80·18*	70·12	67·43	**68·77**	*65·74*	*66·22*
6 inclusive	80·98	81·63	**85·92**	*85·29*	*83·38*	73·14	72·52	**72·83**	*70·43*	*68·86*
6 exclusive	89·85	91·20								
7	102·98	101·18	**102·08**	*88·13*	*84·86*	76·81	75·18	**76·00**	*72·78*	*70·08*
8	98·13	102·18	**100·15**	*89·55*	*85·56*	69·79	80·90	**75·35**	*73·95*	*70·66*
9	96·34	94·26	**95·30**	*90·26*	*85·91*	76·02	69·18	**72·60**	*74·54*	*70·94*

the estimate is $D = H = 86{\cdot}24$ in A and 71·22 in B. The values expected for the covariances of each generation on the basis of uncomplicated mendelian inheritance can then be found by substitution for D and H in the standard formulæ (table 6).

The covariances observed and expected with and without dominance are compared for series A and B in fig. 9. It will be seen that the difference between the expectations obtained assuming $H = 0$ and $H = D$ is so small as compared with the difference between observation and expectation that dominance cannot be regarded as having an effect of any importance on our interpretation of the results. In general the observed values are lower than those expected in the early generations and higher than in the later ones. There is a general similarity between series A and B, though the agreement between expectation and observation is much closer in B than in A. The departures from expectation in both lines are the opposite of those seen in the mean variances, being such as would indicate either repulsion linkage or selection favouring heterozygotes. It is, however, very doubtful whether the departures in B can be regarded as having

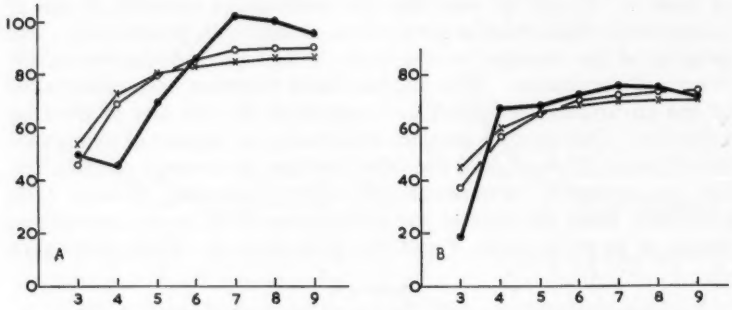

FIG. 9.—Covariance of Parent and Progeny (weighted data). Heavy line, observed values; thin lines, expectation with $H = 0$ (open circles) and $H = D$ (crosses).

any significance. Those in A are more definite in indicating repulsion linkage or selection for heterozygotes, since the graph of observed covariances even crosses that of the expected between generations 5 and 6 as would then be anticipated (see fig. 4). We shall examine the nature of the departure more closely below.

The variances of family means give information very similar to that from the covariances, and their changes must indeed be highly correlated with those of the covariances. No great additional weight can therefore be given to any conclusions in which the variances of means support the covariances.

The variances of means contain, in addition to D and H components, an item E_2 which measures the non-heritable variation of family means. As with E_1, it will be assumed that E_2 was constant over generations. It will also be assumed that $H = 0$. We have already seen that dominance has little effect on the interpretation of the covariances and its effect will be even less on that of the variances of means, for the H component of these is always half that of the

covariances. The assumption of absence of dominance also has the effect of lightening the calculations.

Assuming $H = 0$, the sum of all seven variances of means in either line (weighted for loss due to infertility) will exceed the sum of the seven covariances by $7E_2$. Then assuming $D = 90{\cdot}97$ and $75{\cdot}13$ as

TABLE 7

Variance of means (weighted data)

Generation (F)	Series *A*				Series *B*			
	Observed			Expected when $H = 0$	Observed			Expected when $H = 0$
	Duplicates		Mean		Duplicates		Mean	
3	47·75	42·82	45·29	*49·17*	21·78	8·20	14·99	*40·17*
4	65·78	55·13	60·46	*71·91*	67·24	64·67	65·95	*58·96*
5	69·97	65·44	67·71	*83·28*	93·96	84·32	89·14	*68·35*
6 inclusive	97·28	98·85	103·22	*88·97*	78·27	74·77	76·52	*73·04*
6 exclusive	106·92	109·86						
7	99·16	93·67	96·42	*91·81*	86·38	80·83	83·60	*75·39*
8	104·28	105·47	104·87	*93·23*	71·29	81·82	76·56	*76·56*
9	95·26	93·43	94·35	*93·94*	67·26	59·39	62·83	*77·15*

found from the covariances for series *A* and *B* respectively, E_2 will be estimated as $3{\cdot}68$ in *A* and $2{\cdot}61$ in *B*. These estimates are in reasonable accord with those found for E_1 from the mean variances

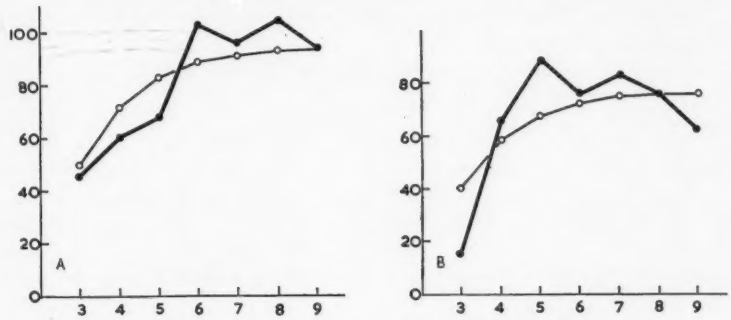

FIG. 10.—Variance of Means (weighted data). Heavy line, observed values; thin line with open circles, expectation when $H = 0$.

and also with the direct estimate of E_2 found earlier. Then substituting for D and E_2 the expected values for the different generation are found as shown in table 7. They are compared with observation in fig. 10.

As would be expected, fig. 10 agrees quite well with fig. 9 which sets out the covariance results. The agreement of expectation and observation in series *B* is less good than in the covariances, but since

the main difference is due to the *fall* observed in the variance of means from F_7 to F_9, no great weight can be attached to it : it must be largely due either to vagaries of sampling or to non-genetic causes. Furthermore, this difference between the indications from the covariances and variances of means is sufficiently large for it to suggest that the apparent contradiction between the mean variances and covariances should not be regarded as having any serious genetical implications.

The behaviour of the covariance and variance of means has been examined further using the figures unweighted for loss of families arising from infertility. In this analysis series *A* and *B* are broken down each into its constituent lines (table 8). Since the covariance

TABLE 8

The mean of the variance of F_n means and covariance of F_{n-1} parents and F_n progenies, within and between lines (unweighted data)

n		3	4	5	6	7	8	9
Within Lines	A 4	...	1·60	0·91	1·01	0·11	0·84	0·21
	A 5	...	12·79	14·76	10·72	0·90 *	0·86	1·84
	A 8	...	4·34	3·42	10·28	8·51	9·46	11·92
	A 9	...	1·48	0·45	9·74	14·34	20·80	27·82
	A10	...	1·65	19·24	44·92	3·05 *	2·98	6·80
	B 1	...	50·25	48·11	37·74	41·92	46·92	41·27
	B 5	...	10·64	11·12	17·51	22·58	18·04	18·62
	Mean	...	11·82	14·00	18·85	13·06	14·27	15·49
Between Lines		39·19	50·81	66·65	79·34	100·60	101·54	95·48

* The sudden fall from the previous generation in these lines is due to the loss of all families with low means.

and the variance of means are so highly correlated they have been averaged for each generation, and the average used in the analysis.

The covariance (and variance of means) calculated between lines, *i.e.* found as the covariance of line means in successive generations (statistics (iii) and (iv) see table 1), should be virtually constant. The coefficient of *H* is reduced to a quarter by each generation but that of *D* is constant at $\frac{1}{2}$. When we come to examine the results, however, we find that the covariance and variance of means are both rising. They average 39·19 for F_2—F_3, rising to 50·81 for F_3—F_4 and reach a peak of 101·54 in F_7—F_8. This must be a reflection of the selection against intermediate phenotypes which we have already observed to be causing the line means to diverge in the later generations. Indeed, the rising covariance and variance of means are themselves clear evidence of such divergence.

The covariance and variance of means within lines, and averaged over lines (statistics (iii) and (v) in table 1) rises more slowly (table 8).

The rise is indeed irregular, there being a sharp peak at F_5—F_6. This is largely due to the irregular behaviour of lines A5 and A10 which both show a sharp fall between that generation and the next, due to death of low-meaned families in each line. If these lines are eliminated the results are less irregular, but even so there is a fall where the greatest rise is expected, between the F_3—F_4 and the F_4—F_5 figures. Thereafter the combined statistic follows much the expected course, perhaps with a tendency to rise too slowly in the way that would follow from repulsion linkage or selection for heterozygotes, herein imitating the much greater deviation of the between-lines statistics (table 9 and fig. 11).

TABLE 9

Mean of covariance of parent and progeny and variance of mean within lines (unweighted data and omitting lines A5 *and* A10)

F	Composition	Observed	Expected	
			$H = 0$	$H = D$
4	$\frac{1}{4}D + \frac{3}{64}H + \frac{1}{2}E_2$	13·66	10·61	11·75
5	$\frac{3}{8}D + \frac{9}{256}H + \frac{1}{2}E_2$	12·80	14·92	15·48
6	$\frac{7}{16}D + \frac{21}{1024}H + \frac{1}{2}E_2$	15·26	17·08	17·05
7	$\frac{15}{32}D + \frac{45}{4096}H + \frac{1}{2}E_2$	17·49	18·16	17·77
8	$\frac{31}{64}D + \frac{93}{16384}H + \frac{1}{2}E_2$	19·21	18·70	18·11
9	$\frac{63}{128}D + \frac{189}{65536}H + \frac{1}{2}E_2$	19·97	18·97	18·27

The expectations are based on $E_2 = 3{\cdot}95$ (estimated by comparison of Covariance and Variance of Mean), $D = 34{\cdot}51$ (when $H = 0$) and $D = 32{\cdot}91$ (when $H = D$) estimated by least squares. The expectations are not substantially altered when $E_2 = 2{\cdot}78$ as found by the direct comparison described earlier.

Any such slight departure of the course of the series of covariances, etc., within lines is, however, small as compared with the effects of the rise in the covariance between lines, especially as there are chance irregularities in it. It is this which caused the major confusion in the weighted analyses. The apparent evidence for repulsion linkage or selection for heterozygotes should not therefore be taken seriously. Another comparison indeed suggests the kind of effect we have already encountered in the mean variances. The minimal value for the average of covariance and variance of means between lines is 39 in F_2—F_3 (table 8). This will be a reasonably unbiased estimate of $\frac{1}{2}D + \frac{3}{32}H$ in that generation, as the non-heritable component will be $\frac{E_2}{2}$. Thus D must be in the neighbourhood of 78, or perhaps a little higher,

in that generation. The covariance between lines would give a higher value for D in later generations. The covariance, etc., within lines, on the other hand, gives an average of about 35 for D in the generations after F_2—F_3. Thus, on this evidence, D has fallen in the way that would arise from coupling linkage or selection for homozygotes.

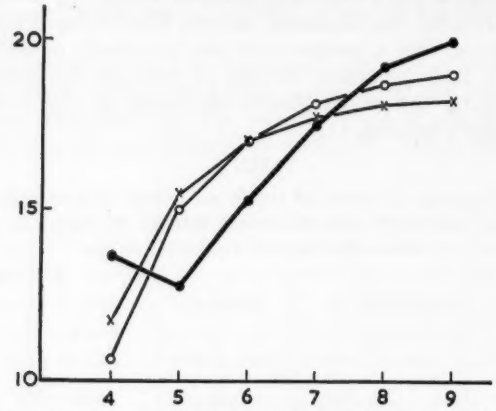

FIG. 11.—Mean of Covariance and Variance of Means within lines, excluding A5 and A10 (unweighted data). Thick line, observed values ; thin lines expectation with $H = 0$ (open circles) and $H = D$ (crosses).

Table 10 shows the changes in the correlation coefficient of parents and offspring over the generations in each series. The correlation coefficient for F_n is found as $\frac{W_n}{(V_{n-1}\,V_n)^{\frac{1}{2}}}$, where W_n and V_n are the covariance and variance of means respectively in that generation,

TABLE 10

Correlation of parent (F_{n-1}) *and offspring* (F_n)

F	Series *A*			Series *B*		
	Duplicates		Mean	Duplicates		Mean
3	0·89	0·89	0·89	...	...	...
4	0·87	0·94	0·91	0·90	0·95	0·92
5	0·91	0·93	0·92	0·92	0·94	0·93
6 inclusive	0·94	0·94	0·94	0·94	0·95	0·94
6 exclusive	0·95	0·95				
7	0·95	0·96	0·96	0·95	0·96	0·95
8	0·95	0·98	0·97	0·91	0·92	0·92
9	0·94	0·93	0·94	0·92	0·90	0·91

and V_{n-1} the variance of means in the previous or parental generation. Now apart from E_2, W_n must closely approximate to V_n as the D components are identical and the H are small. Furthermore, V_{n-1}

differs from V_n only by $(\frac{1}{2})^{n-2}$ in D and not at all in E_2. We should thus expect to find only small changes in the correlation coefficient, changes which will reflect only feebly the rise of the heritable component of variation in V and W relative to the non-heritable component. The coefficients observed fully substantiate this expectation. As will be seen from table 10, the correlation coefficient is uninformative.

Viewing the experiment as a whole, it shows a fair consistency in most respects. The values of E_1 found from the mean variances of the two lines in the weighted data agree well enough with one another, as do the values of E_2 found directly and from the covariances and variances of means. Furthermore, E_1 and E_2 show a reasonable relation with one another.

Table 11 shows the estimates of $D+\frac{1}{2}H$ obtained from the various sources in both weighted and unweighted data. Series *B* seems to have been, by chance, little if any less variable than its ancestor

TABLE 11

Comparison of the estimates of the components of variation $(D+\frac{1}{2}H)$ *from various sources*

Weighted data

Series	Mean Variance (F_3-F_9)	Covariance ($F_{2/3}$-$F_{3/9}$)	
		$H=0$	$H=D$
A	61	91	129
B	71	75	107

Unweighted data (*both series pooled*)

Overall Mean Variance		Covariance and Variance of Means (joint estimate)			
		Between Lines ($F_{2/3}$)		Within Lines ($F_{3/4}$-$F_{3/9}$)	
F_2	F_3-F_9	$H=0$	$H=D$	$H=0$	$H=D$
ca 100	60	78	94	35	59

series *A*. It is thus legitimate to pool the two. It will be seen that whether the estimate comes from mean variance or covariance and variance of means, it is always higher when the F_2 variance, or its equivalent, the F_2—F_3 covariance and F_3 variance of means, is taken as, or included in, the source, than when estimation is solely from the later generations.

With uncomplicated inheritance the various estimates should agree apart from sampling variation ; but a difference of the kind found

is to be expected with either coupling linkage or selection favouring homozygotes. With coupling linkage the values of D and H (as opposed to their coefficients) diminish steadily with the generations, the greatest fall being from F_2 to F_3 (Mather, 1949*a*). A corresponding effect follows when selection favours homozygotes. The coefficients of D and H (as opposed to their actual values) will then fall much more sharply than would be the case in the absence of selection. Then assuming the unselective coefficients of D and H, as we have done in these analyses, the estimates of $D+\frac{1}{2}H$ will fall with the generations.

This fall is directly observable from the analysis of the unweighted data in table 11 where the estimates from F_2 and from later generations are separated ; it being borne in mind, of course, that the F_2—F_3 covariance and the F_3 variance of means depend on the same D and H components as does the variance of F_2. In the weighted data the fall with generations is not shown directly but is nevertheless clear. For the estimate from the mean variance will depend on the values of D and H in generations F_3 onwards, whereas the chief items in the overall covariance must arise from the D and H of F_2 which makes the chief contribution to the overall covariance in all generations. The higher estimate of $D+\frac{1}{2}H$ from the overall covariance as compared with mean variance thus reveals the greater values of D and H in F_2 than in later generations.

Considering next the unweighted data, the value of $D+\frac{1}{2}H$ found from F_2 mean variance is based on but few degrees of freedom and so cannot be regarded as highly reliable. The corresponding estimate from the covariance and variance of means is based on no more degrees of freedom but includes data from the next generations. The general agreement between the mean variances and the other statistics must thus be our chief source of confidence in the trustworthiness of the comparisons and the reliability of the conclusions.

There is thus good overall evidence for the supposition that coupling linkage or selection in favour of homozygotes is operating in the experiment. It may be noted too, that Mather and Philip (*ibid.*) record evidence for coupling linkage in this barley cross, the possibility of selection not being considered. Certainly the results cannot be taken as indicating any great favouring of heterozygotes by selection, such as seems to be found in many species like maize, pigs or poultry, which, we observe, differ from barley also in their natural breeding systems, being out-breeding as opposed to inbreeding.

No analytical means exists at present for using the data of this experiment to decide between the alternatives of coupling linkage and selection favouring homozygotes. No observation has, however, been recorded which would suggest that homozygotes as such are more vigorous or fertile than individuals homozygous for fewer genes in barley, though we have evidence that selection has acted against intermediate phenotypes. It seems unlikely, therefore, that selection

is the explanation of the fall in the components of variation with the generations. Linkage preponderantly in the coupling phase is the more attractive explanation, with little, if any selective differential between the more homozygous and the more heterozygous. The progress of inbreeding, *i.e.* the rise of homozygosis, seems to have followed much the course that would be expected on simple mendelian grounds.

Before leaving this barley experiment it will perhaps be useful to draw specific attention to its three chief defects. The first is that it started with very few F_2 plants (or F_3 plants in series *B*) : the initial sampling variation, therefore, may well have been unduly high. Secondly, as we have already observed, the duplication in the experiment was not such as would provide a valid estimate of error variation. Thirdly, no means was available of independently detecting any changes in the value of E_1 from year to year. In designing future experiments for the measurements of progress in homozygosis under inbreeding, it would be desirable to commence with a bigger population of F_2 individuals, say 20 as a minimum ; to arrange the internal duplication of the experiment in such a way that it would yield a measure of the total sampling variation, that of parents as well as of progeny ; and to include every year the true breeding parent lines at least, so as to provide annually a direct estimate of E_1 and E_2 and thereby to detect any major changes that they might show and that might affect the comparisons upon which judgment of the progress of inbreeding must depend.

5. SUMMARY

The rise of homozygosis under a system of inbreeding will be affected by selection, natural or artificial. It will be slowed down by selection in favour of heterozygotes, and in an extreme case may be prevented altogether. Expected frequencies of homozygosis, calculated on the assumption of inheritance uncomplicated by selection, will not therefore be realised in species where selection is operative. It is to be expected that selection will favour heterozygotes in species, whether plant or animal, which reproduce by outbreeding. It will be of lesser effect, and perhaps absent, in species (such as barley) which normally inbreed.

The biometrical analysis of continuous variation affords an experimental method of studying the progress of inbreeding and the action of selection on it. The different variances and covariances measuring variability in the various generations can be expressed in terms of the quantities *D*, *H* and *E* (Mather, 1949*a* and *b*). The coefficients of *D* and *H* vary with the system of breeding, and are also affected by selection in favour of heterozygotes or homozygotes. *D* and *H* themselves may change in value if breakable linkage is operative. Thus, in theory, linkage and selection have different effects, but, in practice, selection favouring heterozygotes may be

difficult to distinguish from repulsion linkage, and selection favouring homozygotes from coupling linkage.

An experiment with ear size in barley is described in which, following a varietal cross, self-pollination was practised up to F_9. There is some direct evidence of the action of natural selection in the experiment, but it was selection against intermediate phenotypes rather than against heterozygotes or homozygotes. There is also evidence that the scale of measurement of ear internode length was not fully adequate, in that non-heritable variance was correlated with mean measurement. These two disturbances cause certain complications in the analysis and interpretation of the results.

In the absence of selection and linkage the heritable portion of the mean variance of families should be halved in each generation. The series observed suggests that the decline is, in fact, at first more rapid than this. Such a result suggests, either selection for homozygotes, or coupling linkage. The series of parent offspring covariances and variances of family means at first suggests the opposite. Much of this appearance arises from the selection against intermediate phenotypes and, in fact, these statistics cannot be regarded as disagreeing with the mean variances.

When analysed, assuming coefficients uncomplicated by selection, both mean variances, on the one hand, and covariances and variances of means on the other, give values of D and H which are higher in F_2 and F_3 than in later generations. This effect would follow either selection for homozygotes or coupling linkage. It is considered that coupling linkage is the more likely explanation. Certainly there can be no selection favouring heterozygotes, such as would be expected in outbreeding species, though not in barley and other inbreeders.

The parent offspring correlation coefficient is shown to be an uninformative statistic. Certain weaknesses in the design of this experiment are discussed and suggestions made for improvement in future experiments.

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A QUANTITATIVE STUDY OF TWO POPULATIONS OF THE MOTH *PANAXIA DOMINULA* (L.)

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1. INTRODUCTION

BETWEEN 1939 and 1946 an intensive study of an isolated population of the moth *Panaxia dominula* (L.) was carried out by Fisher and Ford (1947) at Cothill. They were able to estimate the number of moths flying in the colony from day to day, as well as the total population size for the season. Moreover, because the seasonal population size was known, they were able to demonstrate that the observed fluctuations in the frequency of a gene, which showed no dominance, were too great to be ascribed to random sampling in the reproduction of the population, a process which is often referred to as " genetic drift." Wright (1948) has criticised their results on the grounds that no population size was calculated for 1939 or 1940. However, the population in 1940 would have to have been as small as the number of moths actually caught to invalidate their results. Even if collecting had been carried out on every day of the season, the population could not have been less than 450 since the data for other years show that they never caught as much as a quarter of the population in the season.

In the winter of 1948, when looking for larvæ of *P. dominula* in all the suitable areas in the vicinity of Cothill, to see if there were any populations which had been overlooked by entomologists, I found that there was a small colony still in existence in Tubney Wood, where the moth was supposed to be extinct (Kettlewell, 1942). I also found a large colony at Sheepstead Hurst, just over a mile down the stream which runs through Cothill. Because very much more information can be obtained by the comparison of the ecology of two or more populations, than by the intensive study of one, it was decided

to investigate the new colony at Sheepstead Hurst as well as the original one at Cothill. It was impossible to study adequately all three breeding-communities at the same time.

The purpose of this paper is to give an account of the results obtained from the continued study of the colony of *P. dominula* at Cothill and a comparison of this population and the newly discovered one at Sheepstead Hurst.

2. A DESCRIPTION OF A NEW VARIETY AND SOME NEW OBSERVATIONS ON THE BEHAVIOUR OF THE MOTH

A detailed description of *P. dominula* and its habits has been given by Kettlewell (1942) and a comprehensive summary of his conclusions, with special reference to the situation at Cothill, may be obtained from the paper by Fisher and Ford (1947). However, since 1946 a new form of the moth has been discovered and more observations have been made on the habits of *P. dominula*. In 1948 a variety of the moth, which I shall call var. A for convenience, was discovered in copulation with a normal female at Cothill. It has the elongated black spot near the anal angle of the hind-wing split into two spots and the area covered by the white and yellow marks on the fore-wing slightly increased. A brood from this female of the new form was successfully raised. It produced seven normal males, eleven normal females and five males and seven females of var. A. No specimens of the new form were found in any other broods raised from normal wild females nor in any other stocks of *P. dominula* kept under similar conditions in the laboratory. The only pairing of the form var. A from which a brood was successfully raised in 1950, was produced by a female which mated with a normal male from another stock. Twenty-four moths emerged in July 1950 and all of them were normal. In the collections of the Hope Department of Entomology at Oxford there are five normal insects which were raised from a pairing of two moths, one of which was var. A. In the Tring collection there is an example of the new form from Winchester, where most of the moths are var. A, which had paired with a normal *P. dominula* from Cothill. There are a large number of offspring from this mating all of which are var. A. However, it is not known if all the progeny of this pairing or the one in the Hope Department are present. It seems certain that the new form is inherited, perhaps as a simple recessive, but it must be remembered that temperature has a considerable effect on the amount of black pigment deposited on the wings of *P. dominula*. It is possible therefore, that the expression of the inherited factors responsible may be modified by the environment. Breeding experiments are being continued to elucidate these points. It has been possible to record the percentage of var. A in the Cothill colony in 1948, 1949 and 1950, as well as the proportion in the Sheepstead Hurst population in 1949 and 1950. A few intermediate

forms between var. A and typical *P. dominula* are found, therefore the arbitrary decision was made that if one of the spots on the two hind-wings was split the moth was recorded as var. A (table 9). The proportions of the new form in the two colonies are different, although whether the difference is purely genetic or both environmental and genetic, is not yet known. In the Cothill population some specimens of var. *medionigra* Cockayne, are also var. A.

It has been stated by Fisher and Ford (1947) that the moth usually emerges from the pupa in the morning and that many of the females pair at once. However, from observations at Cothill and Sheepstead Hurst as well as from breeding experiments in the laboratory, it appears that females do not start assembling males in large numbers before about 2.30 p.m. (G.M.T.) and that they may continue until dusk. It follows that the majority of them must mate in the afternoon as males are not attracted after copulation has taken place. In the laboratory even those females which have emerged some days previously, do not pair readily until the afternoon on the day on which they are put into a cage with males. Kettlewell (1942) believes that females do not fly until the day after copulation and that because they start laying eggs soon after pairing they must lay a considerable proportion of their eggs in the vicinity of the food plant on which they themselves had fed as larvæ. Seven females which had been found mating at Cothill were given a distinctive mark and released in the normal way (page 363). The position in which they came to rest was carefully noted and a thorough search of the area was made the next morning. No female was ever recaptured in this area, although twice a female was caught in a different place. The method of release might upset the normal behaviour of the insect, consequently, the same marking procedure was carried out at Sheepstead Hurst, without disturbing the moths. Six different females found in copulation were used and again none of them was discovered in the same place on the next day. Moreover, fifty females from wild larvæ, which paired on the same day that they emerged were kept in the laboratory in 1950. They were put in separate boxes, which were all about the same size, and shape, and maintained at a temperature of about 60° F. The number of eggs laid each day was recorded together with the length of life of the insect. It was found that females seldom deposited many eggs until the day after copulation and that in the first twenty-four hours they only laid a small proportion of the total number of eggs. However, it must be remembered that the moths were in artificial conditions and that their expectation of life was far higher than in the field.

A summary of the results of this experiment is given in table 1. A significant correlation with $P<0{\cdot}001$, was found between the length of life of females and the total number of eggs deposited (table 2). Even if there are other factors effecting the death-rate in the wild there must still be a correlation between the length of life of a female

and the number of eggs laid. Consequently, the death-rate of adults must be one of the factors, although probably a minor one, in controlling the change in population size from year to year. Among

TABLE 1

Periods of 24 hours after copulation	Number of insects alive	Eggs deposited	Percentage of total number of eggs deposited by the population
1	47	2,394	18·2
2	46	1,812	13·7
3	45	2,248	17·1
4	44	1,707	13·0
5	44	1,341	10·2
6	44	883	6·7
7	42	994	7·5
8	42	618	4·7
9	40	394	3·0
10	34	348	2·6
11	31	223	1·7
12	25	118	0·9
13	22	77	0·6
14	12	15	0·1
15	7	9	0·1
16	1	0	0·0
		13,181	100·1

TABLE 2

Total number of eggs deposited by individuals

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
												214			
												216			
												228			
												251			
								88		293		264		209	
								136		296		344	313	295	
								179		303		345	370	359	
								298	114	309	298	360	373	369	
5	178	20	...	...	87 118	...	257 353	301 383	154 366	315 318	308 344	369 378	444 453	430 447	329

Total Days Survived

the moths investigated, there were three abnormal individuals two of which laid very few eggs in the first seven days and produced no fertile eggs. They both lived eighteen days which was longer than any of the others survived except one which deposited no eggs at all and lived twenty-nine days. The results from these three aberrant individuals supports the view that moths which cannot lay fertile eggs have a greater expectation of life than those which have mated successfully and that therefore their chance of a fertile mating at a later date is increased.

All the evidence suggests that the females do not lay a large pro-

portion of their eggs in the vicinity of the plants on which they themselves fed as larvæ, but that the majority are deposited in other areas of the colony. During the breeding experiments it was discovered that the males very frequently pair several times (one moth paired ten times with different females) and that the females all deposit their normal number of fertile eggs. On the other hand, females were never observed to pair more than once, except very rarely at the end of their lives, when they had started to lay infertile eggs or when they had previously copulated unsuccessfully. The ability of the males, but not the females, to pair on a large number of occasions is of considerable importance in understanding the significance of changes in the sex-ratio during the season (page 372).

Fisher and Ford (1947) have suggested that the pattern on the fore-wings of *P. dominula* makes them rather inconspicuous, especially when they are resting on the flower heads of Hemp Agrimony, *Eupatorium cannabinum* L., and they point out that when the moths are touched they often display the scarlet hind-wings and produce two large drops of amber coloured fluid from the prothorax. However, they did not draw attention to the fact that when they are flying, with their characteristic undulating flight, the scarlet hind-wings produce a flashing effect which makes it very difficult to follow their line of flight. When they suddenly land there is a typical flash colouration effect. In fact this species does not only show cryptic colouration of the ruptive type and warning colouration when violently disturbed but also flash colouration. In this connection it is of interest that on several occasions dragon flies have been seen to catch the moths and then drop them almost immediately. The few times the moths could be discovered in the long grass where they had fallen, they were never found to be severely damaged.

Fisher and Ford (1947) investigated the fluctuations in the frequency of a gene which shows no dominance and, except as a great rarity, is found only in the colony at Cothill. The heterozygotes are known as var. *medionigra* Cockayne and homozygotes as var. *bimacula* Cockayne. Kettlewell (1942) states that in the breeding cage preferential pairings take place between individuals carrying this gene. However, there is no evidence of this from the field, judged by the ratio of the three phenotypes, taking into account their relative selective advantages (page 359). In fact less var. *bimacula* have been found than would be expected if their selective disadvantage is as great as the square of the selective value of the heterozygote. However, this is not conclusive, since it might be greater than this value. Kettlewell's evidence is not based on any strict numerical data but only on observations in breeding cages containing a large number of individuals. In these cages any one pairing will alter the ratio of the genotypes which can pair in the future. It was therefore decided to make a number of similar cages and to put three moths in each, two being of one sex, and one of the other. If two of the moths are var. *medionigra* and the

third typical *dominula* or two *dominula* and the other *medionigra*, four combinations can be obtained.

(*a*) One female *dominula* and one female *medionigra* with one male *dominula*.
(*b*) The same as (*a*) with a male *medionigra* substituted for the male *dominula*.
(*c*) A male *dominula* and a male *medionigra* with a female *medionigra*.
(*d*) The same as (*c*) with a female *dominula* substituted for the female *medionigra*.

Consequently, in each cage either the male or the female had a "choice" of mates. If there is no differential pairing there would be an even chance of either mating taking place first. The results of the first pairings are as follows :—

	dominula × *dominula*	*dominula* × *medionigra*	*medionigra* × *medionigra*
(*a*)	1	2	...
(*b*)	...	3	1
(*c*)	...	3	2
(*d*)	5	6	...

Different varieties pairing, 14 ; same varieties pairing 9.

No tendency for similar varieties to pair with one another has been demonstrated, but the experiment must be considerably enlarged before really useful results will be obtained. However, there should have been some indication of any tendency for preferential matings which was large enough to be detected, with any certainty, by casual observation. In fact the discrepancy from the expected one to one ratio is in the opposite direction, although there is no suggestion of it being significant $P > 0{\cdot}20$.

3. THE SHEEPSTEAD HURST COLONY AND CHANGES IN THE COTHILL COLONY

The population at Sheepstead Hurst, which was discovered in the winter of 1948, occupies a damp wood just over a mile south of the village of Cothill and on the same tributary of the Ock which drains the Cothill marsh. Agricultural land borders the stream both north and south of the colony and also surrounds the wood. Consequently, the population is well isolated from both the Cothill and Tubney Wood habitats. The wood itself is growing in a peat marsh which resembles Cothill in many respects except that it is much damper and there is always a quantity of stagnant water surrounding the reeds (*Phragmites communis* Trin.) and tussocks of grass in the old peat workings. These pits form open spaces in the wood on its northern border and in these clearings Alder and Birch scrub is growing. *P. dominula* is most abundant in these open spaces and is only slightly less common in the thin belt of marshy grassland to the north and

west of the high hedge surrounding the wood, as well as down the stream on its eastern border. In fact *P. dominula* is common in all areas except the densely wooded part at the southern end of the colony. Large quantities of Comfrey (*Symphytum officinale* L.) grow in the grassy areas surrounding the wood and on the banks of the stream but not in any quantity inside the wood or in the peat workings. On the other hand, the dense masses of *P. communis*

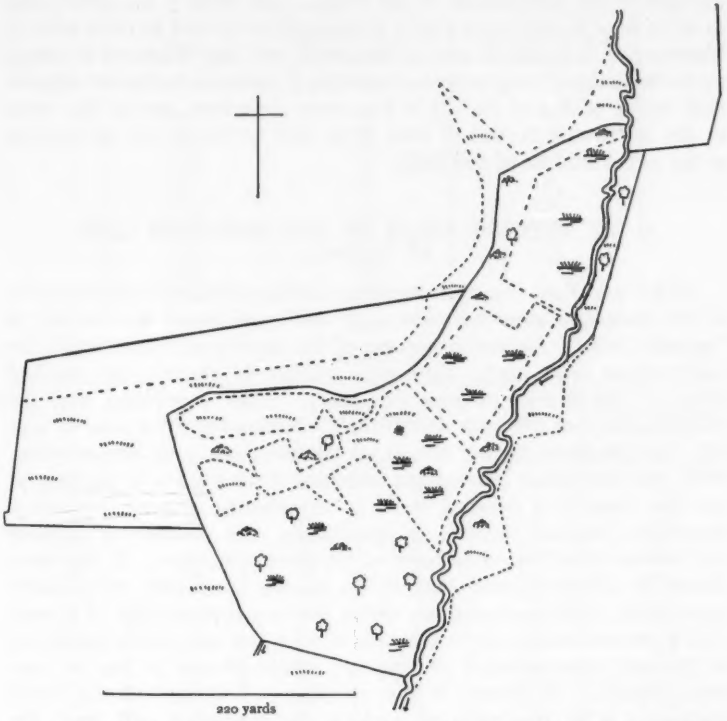

FIG. 1.—In the sketch map of Sheepstead Hurst, the peat workings in the wood are indicated by a broken line. *Panaxia dominula* is common in all areas of the colony except the southern wooded part.

* = releasing point.

are confined to these workings and Hemp Agrimony (*Eupatorium cannabinum* L.) to the thin areas of wood which separate them. The locality in which the new population lives is more wooded and damper than that at Cothill, which was described by Fisher and Ford (1947), but covers about the same area (approximately twenty acres in all (fig. 1).

Since 1946 certain changes, which are worth recording, have

taken place in the Cothill colony. The wood between B and E (see Fisher and Ford, 1947, fig. 1) was felled in 1947 and new scrub about six feet high is growing up from the old stools. *P. communis* is extending its range from area B and is now to be found in the north-west corner of A as well as in large quantities in the rectangular field north of B and D. It has also increased in abundance in area D. The distribution of *P. dominula* in the colony has changed quite markedly in the last four seasons, almost certainly as the result of changes in the distribution of the reeds. The moth is not now found at all in area E and is not nearly so common as it used to be in area C. However, it is common among the reeds and has extended its range up to the limits of the ground occupied by *P. communis* in the rectangular field north of B and D. It is also very abundant among the reeds in the north-east corner of area D as well as being not uncommon as far as the middle of this field.

4. THE SELECTIVE VALUE OF THE *MEDIONIGRA* GENE AT COTHILL

Fisher and Ford (1947) showed that the fluctuations in the frequency of the *medionigra* gene between 1939 and 1946 could not be due to "genetic drift." However, because of the short period over which the observations were made, they were unable to observe any marked trend in the change of gene frequency. They concluded that the selective value of the gene was probably fluctuating from year to year and that the heterozygote was at an advantage to both homozygotes. With the additional information obtained since 1946 it is possible to see that there is a decided trend in the change of gene frequency. Moreover, because there is no dominance it is possible to estimate the relative selective advantages of all three genotypes. It has been shown by Hardy (1908) that in an infinite bisexually reproducing population, mating at random, where the two allelomorphs of a gene, that is not sex-linked, are in the ratio of $p : q$, the ratio of the genotypes in the next generation is $p^2 : 2pq : q^2$, where p^2 and q^2 are the two homozygotes. However, if the selective advantage of the three genotypes is in the ratio of $1 : b : c$ the genotypes will have the frequency $p^2 : 2bpq : cq^2$. Consequently, if the proportions of the two allelomorphs are known, the selective advantage of the three genotypes can be estimated. Ignoring, for the moment, the fact that the population at Cothill is not infinite but is of known size, greater than a thousand individuals, and also ignoring sampling errors in estimating the gene frequency and the ratio of the three genotypes in any season, it is possible to calculate the selective values of the three genotypes for the years 1940 to 1950. If the number of individuals in each genotype is H*c* for the common homozygote, in this instance *dominula ;* H*z* for the heterozygote *medionigra ;* H*r* for the rare homozygote *bimacula* and p represents the normal gene and q its allelomorph,

in the preceding generation, then H*c* is proportional to p^2, H*z* to $2bpq$ and H*r* to cq^2. It follows that :—

$$b = \frac{\text{H}zp}{2\text{H}cq}, \quad c = \frac{Hrp^2}{\text{H}cq^2} \quad \text{and} \quad \frac{b}{c} = \frac{\text{H}zq}{2\text{H}rp}.$$

Table 3 gives the values of *b* and *c* for the different years using the gene ratios obtained by sampling the population (table 4). Fisher and

TABLE 3

Year	Selective value of *medionigra*	Selective value of *bimacula*
1940	1·29	1·06
1941	0·59	0·32
1942	0·82	...
1943	1·11	...
1944	0·81	0·63
1945	1·42	2·72
1946	0·62	0·70
1947	0·85	1·22
1948	0·96	0·74
1949	0·82	...
1950	1·35	...

TABLE 4

Year	*dominula*	*medionigra*	*bimacula*	Total	Gene-frequency per cent.
Up to 1928	164	4	0	*168*	1·2
1939	184	37	2	*223*	9·2
1940	92	24	1	*117*	11·1
1941	400	59	2	*461*	6·8
1942	183	22	0	*205*	5·4
1943	239	30	0	*269*	5·6
1944	452	43	1	*496*	4·5
1945	326	44	2	*372*	6·5
1946	905	78	3	*986*	4·3
1947	1244	94	3	*1341*	3·7
1948	898	67	1	*966*	3·6
1949	479	29	0	*508*	2·9
1950	1106	88	0	*1194*	3·7
	6508	615	15	*7138*	

Ford (1947) point out that no simple relationship exists between population size each season and the frequency of the *medionigra* gene. They suggest that the fluctuations in population size from season to season may be an expression of changes in the direction and intensity of selection. It can be seen that population size can change in a regular manner without a change in the environment and, similarly, the gene frequency can also change regularly. Consequently, if changes in population size are the result of alterations in the direction

and intensity of selection it is necessary to compare change in the rate of change of population size with change in selective advantage. Unfortunately, the estimates of seasonal population size and selective value of *medionigra* are only tentative and therefore little reliance can be placed on such a comparison. However, change in population size, on a logarithmic scale, has been plotted on the same graph as selective advantage of var. *medionigra* (fig. 2). The slope of the lines

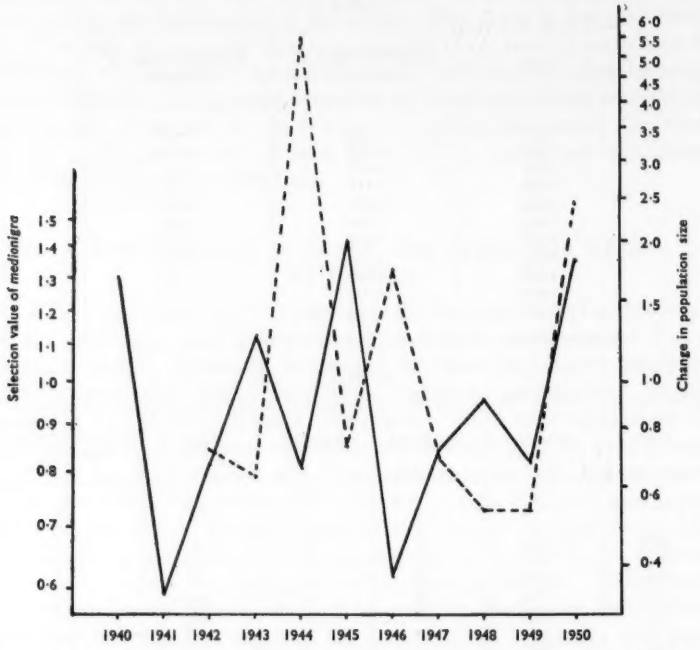

FIG. 2.—In the graph, change of population size is plotted on a logarithmic scale and the slope of the line indicates change in the rate of change of population size. The slope of the continuous line gives the change in the selective advantage of var. *medionigra*.

which indicates change in rate of change of population size and gene frequency go in opposite directions on six out of eight occasions. If there is an equal chance of them sloping either way and it is of equal interest if they always tend to go in the same or opposite directions, the probability of this result being obtained by chance is greater than 0·05 and therefore does not reach the formal significance level. It is possible to make an independent test to ascertain if there is any evidence that the relative selective values of the three genotypes are fluctuating. If the values of b and c are constant it is possible to calculate the expected gene frequency from year to year. The homozygote *bimacula* is rare and therefore makes

little difference to the gene frequency. Consequently, we may assume that the value of c is the same as b^2. From this it follows that the percentage of the gene frequency of the *dominula* gene is $\frac{100p}{p+bq}$, if the ratio of the two allelomorphs in the previous generation is $p : q$.

This simplification makes it possible to use the maximum likelihood method to fit a regression line. If there is no evidence of heterogeneity

TABLE 5

Year	Expected number of *dominula* genes	Expected number of *medionigra* genes	χ^2
1939	408·1	37·9	*0·27*
1940	215·8	18·2	*3·62*
1941	856·5	65·5	*0·11*
1942	383·4	26·6	*0·86*
1943	506·2	31·8	*0·11*
1944	938·5	53·5	*1·43*
1945	707·4	36·6	*3·73*
1946	1883·6	88·4	*0·23*
1947	2572·6	109·4	*0·84*
1948	1860·2	71·8	*0·11*
1949	981·6	34·4	*0·88*
1950	2314·5	73·5	*2·95*
			15·14 $n = 10$

TABLE 6

Year	Expected number of *dominula*	Expected number of *medionigra*	Expected number of *bimacula*	Total
1939	186·7	34·7	1·6	*223*
1940	99·5	16·8	0·7	*117*
1941	397·9	60·7	2·4	*461*
1942	179·3	24·8	0·9	*205*
1943	238·1	30·0	0·9	*269*
1944	443·9	50·7	1·4	*496*
1945	336·3	34·8	0·9	*372*
1946	899·6	84·4	2·0	*986*
1947	1233·8	105·0	2·2	*1341*
1948	895·6	69·0	1·4	*966*
1949	474·2	33·2	0·6	*508*
1950	1121·7	71·1	1·2	*1194*
	6506·6	615·2	16·2	*7138*

in the data, it is unnecessary to vary the value of c to improve the fit. The expected number of *medionigra* for the different years is given in table 6. The method gives a good estimate of the value of b as well as the gene frequency in any year. The value of b is 0·907 that is a selective disadvantage of the heterozygotes of about 10 per cent. The observed frequencies of the *medionigra* gene together with its expected frequencies are plotted in fig. 3. The χ^2 for heterogeneity is 15·14 with 10 degrees of freedom which gives $P > 0{\cdot}10$. It follows

that there is no evidence that the relative selective advantages of the three genotypes are not constant. The expected number of *bimacula* for the years 1939 to 1950 is 16·2, whereas the observed number is 15. This is so close to expectation that it is unnecessary to readjust the value of *c* in order to give a better fit (tables 5 and 6).

The selective value of *medionigra* is particularly interesting not only on account of the increase in frequency of the gene between 1928 and 1939, but also because no marked differential viability between

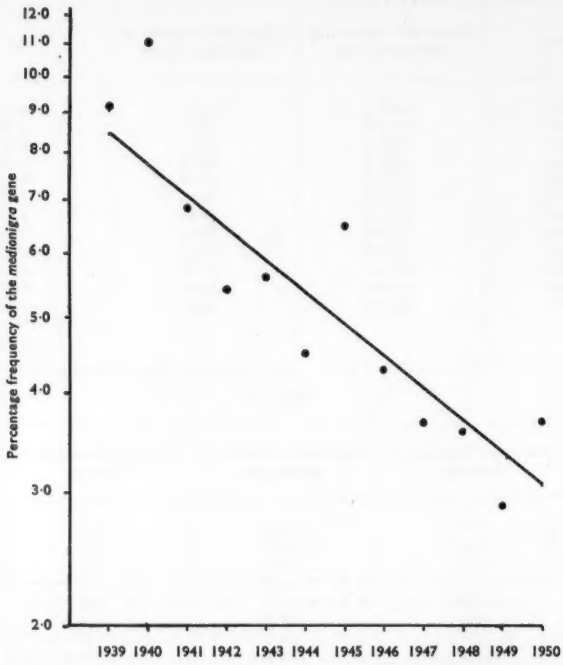

FIG. 3.—In the diagram the observed frequency of the *medionigra* gene is plotted on a logarithmic scale. The regression line has been fitted by assuming that the selective value of the three genotypes are constant.

dominula and *medionigra*, the heterozygote, has ever been observed in the laboratory. Fisher and Ford compared the date of first capture of insects carrying the variant gene and those not carrying it, and found that there was no significant difference between the two. The method they employed has been used to make the same comparison for the seasons 1947, 1948, 1949 and 1950 (table 7). Again there is no evidence of a difference in date of first capture of insects of different genotypes. A further comparison can be made between the behaviour of typical *dominula* and var. *medionigra*. The ratio of the two types released on any day is known as well as their proportions on recapture

on subsequent days. It is therefore possible to compare the expected and observed number of recaptures of the two types (table 8). Although the χ^2 for the difference reaches the formal level of significance in 1947 ($P<0.05$), it is not maintained in the other seasons and over the years 1947 to 1950. The data do not indicate a difference between expected and observed recaptures of individuals carrying the *medionigra* gene.

TABLE 7

Year	Normal gene July	*medionigra* gene July	Difference		χ^2
1947	20·372	19·701	−0·671	earlier	0·1763
1948	20·542	20·647	+0·105	later	0·0387
1949	17·115	15·897	−1·218	earlier	1·2358
1950	19·719	20·409	+0·690	later	0·7777

TABLE 8

Year	Recaptures of *dominula*		Recaptures of *medionigra*		Totals
	Expected	Observed	Expected	Observed	
1947	*230·04*	239	*15·96*	7	246
1948	*270·41*	270	*17·59*	18	288
1949	*160·51*	163	*10·49*	8	171
1950	*407·74*	405	*31·26*	31	439

No *medionigra* or *bimacula* have been observed at Sheepstead Hurst. Thus it is certain that the frequency of the *medionigra* gene is different in the two populations and the gene is probably absent from the new colony. This is important, not only in the light of the other differences between the two breeding-communities, but also because it indicates that there is not a large amount of migration from Cothill to Sheepstead Hurst. Half the moths marked in the new colony were given a distinctive spot and none has ever been recaptured at Cothill, so that migration in the opposite direction is also not very common if it occurs at all. Consequently, it can be said with some certainty that the two populations are well isolated by the agricultural land between them.

The new form of *dominula* (var. A) is found in both colonies and the percentage of this form at Cothill and Sheepstead Hurst from year to year is given in table 9. Not enough information is available to say anything except that the percentage of var. A at Cothill is much lower than in the other colony. The proportion of this form at Cothill increased between 1948 and 1949 and decreased again in 1950, it also decreased significantly between 1949 and 1950 at Sheepstead Hurst. This difference between the colonies is genetic although it may also be environmental. The environment, as far as it affects

the ecology of *P. dominula* in the two colonies, is different. This is clearly shown by the increase in population size at Cothill between 1949 and 1950 and the decrease in the other colony over the same period. Moreover, there is apparently a difference in the death-rate

TABLE 9

Year	Cothill			Sheepstead Hurst		
	Var. A	Total	Per cent. var. A	Var. A	Total	Per cent. var. A
1948	21	966	2·2	...	...	...
1949	23	508	4·5	88	528	16·7
1950	26	1194	2·2	66	935	7·1

of adults in the two colonies although it is not known whether this is significant as it has, so far, been impossible to assign a standard error to the death-rate. Furthermore, in the early part of May 1950, random samples of larvæ were taken from both colonies and the proportion of caterpillars in their last instar in the two populations was found to differ significantly (table 10). This effect is almost

TABLE 10

	Cothill	Sheepstead Hurst
In last larval instar	26	96
Not yet in last larval instar	53	36

certainly environmental as stocks from the two localities reared in constant conditions in the laboratory showed no difference in the time at which they reached the last larval instar. There is therefore a real distinction between the environment in the two areas although they are close together and similar in general features. Seven examples of a pale orange hind-winged form of *P. dominula* have been found at Cothill since 1947 but neither the F_1 generation of one of these moths which paired with a normal form nor the F_2 have produced any examples of this orange variety. Kettlewell (personal communication) has been unable to demonstrate that pink or orange hind-winged varieties of this moth are the result of inherited factors. None of these aberrant individuals has been found at Sheepstead Hurst.

5. THE ESTIMATION OF DAILY AND SEASONAL POPULATION SIZE

To obtain an estimate of daily population size in the two localities, a sample of insects was collected each day. To ensure that this was random, equal areas of the colonies were collected in for equal periods

of time, and all the moths that could be caught were placed in separate boxes and kept until the whole sample had been taken. Each insect was then marked by the method developed by Dowdeswell, Fisher and Ford (1940) using a spot of quick drying cellulose paint which is waterproof and permanent. By varying the colour and position of the mark, it was possible to leave a permanent record on each insect of how many times and on what dates it had been captured. Any individuals which were obviously damaged were killed and recorded as having been killed. All other moths were taken to a central releasing point and were thrown, one at a time, high into the air. Most of them took to the wing and could be seen flying away into the colony. The few moths that failed to fly outside an arbitrary distance of ten yards were again thrown into the air and if they still did not fly they were left until next day. In the morning this area of ten yards radius was carefully searched and any marked insects in it, which still would not fly, were killed, and recorded as having been killed on the date they were captured. Furthermore, no sampling was ever carried out in this releasing area. This ensures that no insects which had been damaged and therefore were incapable of distributing themselves at random in the population were released. Further details of this technique and of the method of estimating population size have been given by Fisher and Ford (1947) and will therefore be only summarily dealt with here. From a knowledge of the time interval between release and recapture of moths recaptured on a particular day it is possible to estimate the death-rate of adults, providing it is assumed that it is a constant. In fact, no significant departure from a constant death-rate could be detected. Fisher and Ford (1947) estimated the best possible death-rate for the years 1941 to 1945 and used it to determine the number of marks carried by insects in the population from day to day. If this is known, then from a knowledge of the number of insects caught on any one day and the number of marks they carry between them, it is possible to estimate the daily population size.

There is no reason to suppose the death-rate in the Sheepstead Hurst colony to be the same as that at Cothill or that it is the same from year to year. Consequently, in this paper, separate death-rates have been estimated for both sexes and both colonies in each successive year. Table 11 gives the death-rate of insects in the Cothill population for the years 1947 to 1950 and in the Sheepstead Hurst colony for 1949 and 1950 as well as the death-rates for both sexes. It will be seen that the estimated expectation of life of females is higher than that of the males in both colonies in 1949 and 1950, and this difference in the new colony in 1949 is large. Also the best estimate of death-rate of each sex in this population has always been lower than that for the corresponding sex in the Cothill colony. An independent check on the difference between the death-rates of the sexes can be obtained by a comparison of the observed and expected number of marks

carried by males and females recaptured on any date. The expected ratio of marks between the sexes among recaptures can be obtained from the estimated number of marks carried by each sex in the population on that day. This ratio may be upset if one sex is more readily captured than the other. A comparison of the results for

TABLE 11

Year	Locality	Sex	Daily survival rate
1947	Cothill	males and females	0·755
1948	,,	males and females	0·729
1949	,,	males and females	0·645
1949	,,	males	0·587
1949	,,	females	0·670
1949	Sheepstead Hurst	males and females	0·722
1949	,, ,,	males	0·600
1949	,, ,,	females	0·804
1950	Cothill	males and females	0·762
1950	,,	males	0·757
1950	,,	females	0·777
1950	Sheepstead Hurst	males and females	0·806
1950	,, ,,	males	0·796
1950	,, ,,	females	0·829
1950	Sheepstead Hurst (without extra mark)	males and females	0·801
1950	Sheepstead Hurst (with extra mark)	males and females	0·811

1949 and 1950 (table 12) shows that the use of a larger death-rate for males has improved the fit three out of four times and that even at Sheepstead Hurst in 1950 when there was no improvement, the difference is not nearly significant. In 1949 the difference in the

TABLE 12

Year	Locality	Recaptures of females		Recaptures of males	
		Expected	Observed	Expected	Observed
1949	Cothill	*101·54*	114	*69·46*	57
1950	,,	*195·43*	199	*243·57*	240
1949	Sheepstead Hurst	*47·14*	43	*25·86*	30
1950	,, ,,	*88·45*	75	*149·55*	163

estimated death-rate of males and females in this colony was large. Moreover, if the same daily survival value of 0·722 is used for both sexes when calculating the expected number of recaptures among males and females, a significant discrepancy between the expected and observed recaptures is obtained. Furthermore, at Cothill in 1949 there is still a large difference between the expected and observed number of recaptures of the two sexes even when the best estimates of daily elimination are used. This evidence strongly suggests that the death-rates of males and females were not the same in 1949.

Before discussing daily and seasonal population sizes it is necessary to consider the effect of the mark on the expectation of life of the insect carrying it. If the expectation of life of moths carrying a mark is different from those not carrying it, the calculation of daily population size will not be appreciably affected because the estimated death-rate is only used to calculate the number of marks carried by insects in the population. However, the seasonal population size will be affected as its calculation must be based on the total number of emergences, which can only be estimated from a knowledge of the death-rate of all the insects in the colony. The effect of marking was tested at Sheepstead Hurst in 1949 and 1950. Approximately half the moths were marked in the usual way and the other half were given an extra mark which was put on the white central spot on the fore-wing (a position never used for date marks). The extra marks were of two types in equal proportions, one white and one blue. The white mark which is on a normally white area, could not be readily seen even on an insect at rest. Precautions were taken to ensure that insects chosen for double marking were taken at random. Boxes containing all the insects which had not been previously marked were placed in a pile, and divided into equal heaps, and one of these was again divided. From these two small piles one was chosen at random and the moths in these boxes received an extra blue mark, whereas those in the other small pile were given an extra white mark. The expected and observed number of recaptures of single and double marked individuals in 1949 and 1950 is given in table 13. It will

TABLE 13

Year	No extra mark		Extra white mark		Extra blue mark	
	Expected	Observed	Expected	Observed	Expected	Observed
1949	*32·39*	33	*15·82*	16	*15·79*	15
1950	*101·88*	85	*51·71*	67	*52·41*	54

be seen that the observed number of recaptures is very close to its expectation in 1949. However, in 1950 a significant excess of double marked individuals was recaptured. The death-rates of double and single marked moths are given in table 11 and are obviously not significantly different. Moreover, the discrepancy between the observed and expected number of recaptures among double marked insects cannot be the result of the observed difference in the daily survival rate of the two types, because it would have to be much larger to account for the disagreement. An alternative explanation is that insects carrying the extra mark are easier to recapture than those not carrying it. Nevertheless, this cannot be

the result of a decrease in the effectiveness of the protective colouration because insects carrying a white mark, which cannot be seen except after close examination, are recaptured as often as those bearing a distinctive blue one. It is therefore probable that the mechanical effect of a small spot of paint on the wings makes the moths easier to catch when flying. This can be tested next season by recording which insects are captured at rest and in flight (see Note p. 378). It is of interest that no discrepancy between the two types of individuals was detected in 1949, when, because of the hot season, the moths were particularly active. The death-rate of adults is known and therefore the frequency of recapture of insects marked more than once in the Cothill population can be examined. This can be done by estimating the number of insects bearing one, two, three and four marks in the population on any day and comparing it with the proportion of these types among recaptures (table 14).

TABLE 14

Year Locality	1 mark		2 marks		3 marks		4 or more marks	
	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
1947 Cothill	*182·42*	176	*22·45*	26	*2·60*	6	*0·53*	0
1948 Cothill	*187·43*	189	*37·75*	43	*10·18*	3	*0·64*	1
1949 Cothill	*105·90*	101	*20·64*	29	*6·38*	4	*1·08*	0
1950 Cothill	*273·51*	279	*62·45*	60	*12·18*	12	*3·86*	1
	749·26	745	*143·29*	158	*31·34*	25	*6·11*	2

Although the data give an indication that there may be a decrease in the expectation of life with an increase in age it will not be large enough to materially alter the estimated population sizes. On the other hand, there seems no very compelling reason for believing that marking increases the ease of recapture. However, even if this were so, Fisher and Ford's conclusions that the fluctuations in the gene frequency of the *medionigra* gene cannot be due to chance, will not be invalidated because their estimated seasonal population sizes will be too small and not too large. Until more data are available, it may be tentatively assumed that marking does not markedly increase the ease with which the insect may be captured. Tables 15 to 26 give all the information necessary for calculating death-rates and population size. This type of table has not been used before and therefore requires some explanation. It is divided into vertical columns each headed by the appropriate date. At their foot will be found the number of moths caught and released each day. The numbers in the body of the table refer to the number of marks retaken at varying intervals of time after first capture. The date to which each refers is given at the

TABLE 15

COTHILL. *Males and females*

July 1947

Days from first release	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1		.	.	.	3	0	5	6	6	6	4	8	8	8	9	3	2
2		.	.	.	.	1	4	7	7	4	3	2	3	11	3	9	5
3				0	.	.	2	2	2	3	2	2	4	5	4	6	5
4					0	.	.	2	2	1	6	3	5	5	7	1	6
5						0	.	.	0	1	4	3	0	1	2	2	3
6							0	.	.	0	0	3	1	2	1	0	2
7								0	.	.	2	0	1	0	3	0	3
8									0	.	.	1	1	1	3	0	0
9										0	.	.	0	0	1	2	0
10											0	.	.	0	0	0	0
11												0	.	.	0	0	0
12													0	.	.	1	0
Captures	.	.	23	.	.	66	89	74	134	132	105	110	115	105	128	174	109	100	82
Releases	.	.	23	.	.	57	82	67	128	116	96	94	109	96	117	158	88	87	76

TABLE 16

COTHILL. *Males and females*

July 1948

Days from first release	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1		0	3	4	2	1	.	.	1	3	1	5	1	2	2	2	6	6	5	7	8	5	5	0	1	4	3
2			1	3	3	1	.	2	.	1	3	0	2	4	2	4	4	5	5	4	2	1	3	5	0	5	1
3				1	0	5	.	2	5	.	1	2	4	0	2	4	6	1	8	5	3	1	1	3	3	2	4
4					0	2	.	1	1	1	.	0	0	1	2	1	1	3	3	4	3	2	2	0	1	1	2
5						1	.	2	1	0	1	.	2	0	3	1	1	2	3	0	0	0	2	2	2	0	1
6							.	1	0	0	0	0	.	0	0	2	1	0	2	0	0	0	0	2	1	0	0
7								2	0	0	0	0	0	.	0	0	2	0	1	0	3	0	0	0	1	1	0
8									0	0	0	0	0	0	.	0	2	0	0	0	1	1	0	0	0	0	1
9										0	1	0	0	0	0	.	0	1	1	0	0	0	2	0	1	0	1
10											0	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0
11												0	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
12													0	0	0	0	0	0	.	0	1	0	0	0	0	0	0
Captures	.	.	2	19	24	31	27	34	.	28	40	31	46	34	35	52	51	72	67	75	94	69	64	51	44	45	63	59	45
Releases	.	.	2	17	23	30	24	33	.	27	40	29	44	32	34	49	46	70	59	67	88	67	60	47	39	39	60	56	42

TABLE 17

COTHILL. *Males*

July 1949

Days from first release	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	.	.	.	.	0	2	1	1	0	0	2	1	2	2	0	0	3	2	1	1	1	1	1	1	.	.
2		0	.	0	.	1	0	1	0	1	0	1	1	0	3	1	0	2	1	0	1	1	1	1	.	0
3			.	0	.	1	0	0	2	1	0	1	0	0	2	1	0	2	0	2	0	0	0	0	.	0
4				0	.	0	.	0	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	.	0
5					0	.	0	.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	.	0
6						0	.	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	.	0
7							0	.	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0
8								0	.	1	.	0	0	0	0	0	0	0	0	0	0	0	0	1	.	1
Captures	2	.	8	.	12	7	30	10	12	6	13	18	26	20	7	24	20	32	14	9	7	8	5	10	.	4
Releases	2	.	8	.	12	7	28	10	12	5	12	16	24	20	7	23	19	30	13	9	7	8	5	10	.	3

TABLE 18

COTHILL. *Females*

July 1949

Days from first release	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	.	.	.	.	0	1	1	0	0	0	0	1	4	3	2	0	4	1	7	2	4	4	0	.	.
2		0	.	0	.	0	1	0	2	0	0	1	1	1	2	2	2	4	5	3	4	3	0	.	1
3			.	.	0	.	1	0	1	0	0	0	0	0	0	1	3	4	0	5	1	3	0	0	.	1
4				0	.	0	.	0	0	1	0	0	0	0	0	0	1	5	1	2	1	2	3	0	.	0
5					0	.	0	.	0	0	0	0	0	0	0	1	0	0	2	1	0	2	0	0	.	0
6						0	.	0	.	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	.	0
7							0	.	0	.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	.	0
8								0	.	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0
9									0	.	0	.	0	0	0	0	0	0	0	0	0	1	0	0	.	0
Captures	4	.	2	.	4	2	7	8	5	6	14	6	10	24	16	16	26	31	27	34	26	28	19	15	.	8
Releases	4	.	2	.	4	2	6	8	5	6	12	6	10	24	15	15	25	31	26	34	26	26	19	14	.	8

TABLE 19

SHEEPSTEAD HURST. *Males*

July 1949

Days from first release	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	1	.	.	.	.	0	0	0	0	1	0	0	2	0	0	0	2	3	1	0	.	.	0	0	.	.
2	2		0	.	1	.	0	0	0	2	0	2	0	1	0	1	0	1	0	0	.	0	.	0	.	0
3	3			.	.	0	.	0	0	1	0	1	0	0	1	1	0	0	0	1	.	0	0	.	.	0
4	4				0	.	0	.	0	0	0	0	0	0	0	0	1	0	0	2	.	1	0	1	.	0
5	5					0	.	0	.	0	0	0	0	0	0	0	0	0	0	0	.	1	0	0	.	.
6	6						0	.	0	.	0	0	1	0	0	1	0	0	0	0	.	0	0	0	.	0
Captures	7	.	11	.	9	2	18	41	25	35	10	26	21	13	18	46	17	18	13	12	.	7	4	7	.	2
Releases	7	.	11	.	8	2	18	40	25	33	9	25	21	12	17	46	16	18	12	12	.	7	3	6	.	2

TABLE 20

SHEEPSTEAD HURST. *Females*

July 1949

Days from first release	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	1	.	.	.	.	0	0	0	0	0	0	0	1	0	1	1	3	0	0	1	.	.	1	0	.	.
2	2		0	.	0	.	0	0	0	0	0	3	1	0	0	0	0	3	0	0	.	2	.	1	.	0
3	3			.	.	0	.	0	0	1	0	2	1	0	0	0	1	0	0	1	.	1	0	.	.	0
4	4				0	.	0	.	0	0	0	0	1	0	0	0	0	0	0	1	.	2	0	0	.	0
5	5					0	.	0	.	0	0	0	0	0	0	0	0	1	0	0	.	0	1	0	.	.
6	6						0	.	0	.	0	0	1	0	0	0	0	0	2	0	.	2	0	0	.	0
7	7							0	.	0	.	0	0	0	0	0	0	0	0	2	.	0	0	0	.	0
8	8								0	.	0	.	0	0	0	0	0	1	0	1	.	0	0	0	.	0
9	9									0	.	0	.	0	0	0	0	0	1	1	.	1	0	0	.	0
Captures	7	.	5	.	5	3	6	5	11	26	6	21	22	21	10	21	19	21	21	28	.	30	21	9	.	3
Releases	7	.	5	.	3	3	6	4	11	26	6	21	20	18	10	20	16	21	21	28	.	30	21	7	.	3

TABLE 21

COTHILL. *Males*

July 1950 — August

Days from first release	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4
1	1	0	0	2	1	0	2	1	8	3	1	2	2	3	5	2	4	2	3	3	3	6	2	2	3	1	1	0	0	0	0
2	2		0	0	0	0	0	3	1	2	1	3	1	3	2	3	2	1	1	1	2	2	0	4	2	1	2	0	0	0	0
3	3			0	0	0	0	2	2	2	4	1	0	2	0	0	0	4	1	0	0	1	2	2	2	0	2	0	0	0	0
4	4				0	0	1	2	0	2	2	2	1	1	0	3	0	1	1	2	3	2	0	3	2	1	0	0	0	0	0
5	5					0	1	1	0	2	2	1	2	2	0	3	0	1	1	3	2	1	0	2	2	1	2	0	1	0	0
6	6						0	1	0	0	0	0	0	3	3	2	0	0	1	2	2	1	0	1	3	0	1	0	0	0	0
7	7							0	0	0	0	0	0	2	7	1	0	0	0	1	0	2	1	0	2	1	1	0	0	0	0
8	8								0	0	0	0	0	1	0	4	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
9	9									0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
10	10										0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
11	11											0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
12	12												0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
13	13													0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Captures	5	5	14	15	20	33	26	83	32	28	40	39	30	48	51	60	43	37	30	37	40	38	17	21	16	8	15	6	6	1	5
Releases	5	5	14	15	20	32	26	83	32	28	39	37	30	48	50	57	41	34	30	36	38	37	16	20	16	8	15	6	6	1	4

TABLE 22

COTHILL. *Females*

July 1950 — August

Days from first release	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4
1	1	0	0	0	0	0	0	0	2	1	1	0	0	1	1	3	2	0	2	1	1	3	0	3	5	2	2	5	1	0	0
2	2		0	1	0	1	1	3	0	2	2	0	0	0	2	0	4	1	2	0	3	3	2	3	0	3	2	0	2	1	3
3	3			0	0	0	2	0	0	0	3	0	1	0	0	1	0	2	1	3	4	4	1	1	4	1	2	1	1	1	1
4	4				0	0	0	0	0	0	0	0	2	2	1	0	1	0	1	2	3	0	1	1	5	0	1	1	1	0	2
5	5					0	0	2	0	0	1	0	2	1	0	0	0	2	0	2	0	2	1	2	3	1	2	0	1	2	0
6	6						0	0	0	0	0	0	0	1	2	2	1	0	0	1	0	2	2	1	1	0	1	1	1	0	2
7	7							0	0	0	0	0	0	0	2	1	0	0	0	0	0	2	1	0	1	0	1	0	0	0	0
8	8								0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
9	9									0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	2	0	1	0	0	0
10	10										0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
11	11											0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
12	12												0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	13													0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	14														0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
15	15															0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	16																0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Captures	1	2	6	9	7	11	11	14	19	17	17	3	25	16	53	53	40	28	24	27	41	39	29	44	47	15	33	21	18	8	19
Releases	1	2	6	9	7	11	11	14	18	17	16	3	24	16	49	51	40	28	24	26	41	39	29	42	47	15	32	21	18	8	19

TABLE 23

SHEEPSTEAD HURST. *Males*

Days from first release	July 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	August 31	1	2
1		0	0	3	2	2	2	5	2	4	1	1	0	1	0	1	0	2	3	1	3	0	0	1	0	0	0	0	0
2			1	0	3	2	1	3	3	3	1	0	3	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
3				0	2	1	1	2	2	0	1	0	0	1	0	1	1	2	1	2	1	1	1	0	0	0	0	0	0
4					1	1	2	4	2	1	1	1	0	0	0	0	0	0	1	0	0	2	1	0	0	2	0	0	0
5						1	0	1	2	2	1	1	4	0	1	2	0	0	0	0	0	0	1	0	0	0	0	0	1
6							1	0	0	0	0	2	2	1	2	0	1	0	0	4	0	0	0	1	3	0	0	0	0
7								0	0	0	2	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0
8									0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0
9										0	0	0	1	0	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0
10											0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
11												0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
12													0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0
13														0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
14															0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15																0	0	0	0	0	0	0	0	0	0	0	0	0	0
16																	0	0	0	0	0	0	0	0	0	0	0	0	0
17																		0	0	0	0	0	0	0	0	0	0	0	0
Captures	19	11	32	36	47	21	30	53	48	35	45	21	39	26	31	29	22	25	26	30	20	18	12	15	6	6	3	1	2
Releases	18	11	31	34	47	21	30	52	47	35	43	21	35	25	31	28	22	24	26	30	20	18	12	15	6	6	3	1	2

TABLE 24

SHEEPSTEAD HURST. *Females*

Days from first release	July 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	August 1	2
1		0	1	0	0	0	1	0	0	0	0	1	0	1	3	1	1	1	0	0	0	3	2	1	0	0	0	0	1
2			1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0
3				0	0	0	0	0	2	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1
4					0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0
5						1	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	1	0	1	0	0	0
6							1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1
7								0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0	0
8									0	0	0	0	0	0	0	0	0	1	0	0	0	1	2	0	0	0	0	0	0
9										0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0
10											0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
11												0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12													0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
13														0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Captures	3	5	7	7	9	9	11	7	16	12	12	21	8	29	28	29	15	21	22	27	19	24	22	16	18	5	20	10	3
Releases	2	5	5	6	9	9	11	7	16	12	12	20	8	27	27	29	15	21	20	27	18	23	22	16	17	5	20	10	2

TABLE 25

SHEEPSTEAD HURST. *Males and females without an extra mark*

Days from first release	July 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	August 1	2
1		0	1	2	2	0	1	1	1	1	1	0	0	1	2	0	0	2	2	1	2	2	0	0	0	0	0	0	1
2			0	0	1	0	1	1	2	1	0	0	0	0	0	0	0	1	0	0	0	2	1	1	1	1	0	0	0
3				0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
4					1	0	0	1	1	0	1	0	0	0	0	0	0	0	1	2	0	2	0	0	0	0	1	0	0
5						1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0
6							1	0	0	0	1	1	0	1	2	0	0	1	0	2	0	1	1	0	0	0	0	0	0
7								0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	0	0
8									0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	1	0	0
9										0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10											0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
11												0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12													0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
13														0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14															0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15																0	0	0	0	0	0	0	0	0	0	0	0	0	0
16																	0	0	0	0	0	0	0	0	0	0	0	0	0
17																		0	0	0	0	0	0	0	0	0	0	0	0
Releases	10	8	18	20	28	13	20	27	30	23	28	19	19	28	29	25	18	22	23	31	18	23	17	14	10	4	10	6	3

top of the column and the time, in days, from its first release on the left-hand side of the table.

The daily population size of a colony can be estimated from the number of marks available for recapture on any day as well as the number of insects caught and marks retaken. However, at the be-

TABLE 26

SHEEPSTEAD HURST. *Males and females with an extra mark*

Days from first release	July 1950 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	August 31	1	2
1		0	0	1	0	2	2	4	1	3	0	2	0	1	1	2	1	1	1	0	1	1	2	2	0	0	0	0	0
2			2	0	2	2	0	2	2	2	1	0	3	0	0	0	1	0	0	0	1	0	0	2	3	1	0	0	0
3				0	2	1	1	2	4	0	0	0	0	0	0	2	0	3	0	1	1	1	0	0	1	0	0	1	0
4					0	1	2	3	1	1	0	1	0	0	0	1	0	0	1	0	0	1	1	1	2	2	1	0	0
5						1	0	1	1	1	0	1	3	0	1	2	1	0	1	0	0	0	0	2	1	0	0	1	0
6							1	0	0	0	1	0	2	0	1	0	1	0	0	2	0	0	0	0	1	0	1	1	0
7								0	0	1	1	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0
8									0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0
9										0	0	0	0	0	1	2	0	0	0	0	1	0	1	0	0	0	0	0	0
10											0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
11												0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0
12													0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
13														0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Releases	. 10	8	18	20	28	17	21	32	33	24	27	22	24	24	29	32	19	23	23	26	20	18	17	17	13	7	13	5	1

ginning of the season when the number of marks in the population is small and at the end of the season when the samples are often small, there are days on which no marked insects are caught. Furthermore, there are some days in the middle of the season when collecting is impossible. It is therefore essential to have a method of estimating an average population size over a number of days so that an idea of the size of the daily population can be obtained for all days in the season. This average population size for a number of days is best obtained by multiplying the estimated number of marks by the size of the sample for separate days, summing them, and dividing this by the sum of marks recaptured over the days in question. This method gives greater weight to the larger samples and to the days when the greatest number of marks were available for recapture. The number of insects in the colony in the last few days of the season can be estimated by taking the average population size for the last three days on which reasonable samples can be obtained and then reducing this from day to day using the known rate of daily elimination. If this is done and if collecting started at the beginning of the season, when very few moths were available for capture, the seasonal population size can be obtained by dividing the sum of the daily population sizes for the whole season by the expectation of life (one hundred divided by the percentage daily elimination of moths). Tables 27 to 32 give the daily population size for the different seasons averaged over short periods (usually three days) as well as the observed sex ratio for 1949 and 1950. Table 33 gives seasonal population sizes for 1947 to 1950. The fact that the sex ratio changes from day to day as judged by daily samples of insects from the colony and estimated population sizes, is of extreme importance because it gives a measure

of the accuracy of the estimates of daily population sizes. This change is also an important part of the breeding structure of the colony. Figs. 4 and 5 show the average daily population size for three day

TABLE 27

COTHILL 1947

Date	Population size
July 14-17	1963
July 18-20	1572
July 21-23	1437
July 24-27	1282

TABLE 28

COTHILL 1948

Date	Population size
July 6-10	145
July 11-13	180
July 14-16	424
July 17-19	459
July 20-22	479
July 23-25	535
July 26-28	600
July 29-31	645

TABLE 29

COTHILL 1949

Date	Insects captured			Estimated daily population size		
	Females	Males	Per cent. males	Females	Males	Per cent. males
July 5-10	23	67	*74·4*	25	120	*82·8*
July 11-13	25	31	*55·4*	64	73	*53·3*
July 14-16	40	64	*61·5*	89	113	*55·9*
July 17-19	58	51	*46·8*	91	128	*58·4*
July 20-22	92	55	*37·4*	90	117	*56·5*
July 23-25	73	20	*21·5*	120	50	*29·4*
July 26-28	23	14	*37·8*	239	29	*10·8*

TABLE 30

SHEEPSTEAD HURST 1949

Date	Insects captured			Estimated daily population size		
	Females	Males	Per cent. males	Females	Males	Per cent. males
July 5-15	110	198	*64·3*	271	424	*61·0*
July 16-18	52	77	*59·7*	918	420	*31·4*
July 19-21	61	48	*44·0*	312	224	*41·8*
July 22-24	58	19	*24·7*	255	83	*24·6*
July 25-28	33	13	*28·3*	808	126	*13·5*

periods at Cothill and Sheepstead Hurst in 1950, where the estimated and observed sex ratios agree moderately well. Although the estimates of daily population size show considerable fluctuations because of

sampling errors, it can be seen that the maximum population size of males is reached before that for females. This ensures that the largest

TABLE 31

COTHILL 1950

Date	Insects captured			Estimated daily population size		
	Females	Males	Per cent. males	Females	Males	Per cent. males
July 6- 8	17	34	*66·7*	72	173	*70·6*
July 9-11	29	79	*73·1*	116	563	*82·9*
July 12-14	50	143	*74·1*	191	355	*65·0*
July 15-17	45	109	*70·8*	148	454	*75·4*
July 18-20	122	154	*56·6*	437	334	*43·3*
July 21-23	92	110	*54·5*	414	569	*57·9*
July 24-26	107	115	*51·8*	305	333	*52·2*
July 27-29	120	54	*31·0*	301	155	*34·0*
July 30- 1	69	29	*29·6*	275	146	*34·7*
Aug. 2- 4	45	12	*21·1*	179	105	*37·0*

TABLE 32

SHEEPSTEAD HURST 1950

Date	Insects captured			Estimated daily population size		
	Females	Males	Per cent. males	Females	Males	Per cent. males
July 6- 9	28	126	*81·8*	109	422	*79·5*
July 10-12	27	104	*79·4*	197	315	*61·5*
July 13-15	40	128	*76·2*	462	493	*51·6*
July 16-18	58	86	*59·7*	699	696	*49·9*
July 19-21	72	82	*53·2*	377	710	*65·3*
July 22-24	70	81	*53·6*	609	466	*43·3*
July 25-27	65	50	*43·5*	416	273	*39·6*
July 28-30	39	27	*40·9*	290	122	*29·6*
July 31- 2	33	6	*15·4*	246	77	*23·8*

TABLE 33

Approximate estimates of seasonal population sizes

Year	Locality	Males	Females	Totals
1947	Cothill	...	...	5000-7000
1948	,,	...	...	2600-3800
1949	,,	938	797	1400-2000
1950	,,	2381	1741	3500-4700
1949	Sheepstead Hurst	2563	2976	5000-6000
1950	,, ,,	2323	1934	3600-4800

number of females are mated in the shortest time. For it will be remembered that the females only mate once but that the males are

capable of copulating successfully with a large number of females. Consequently, the important ratio from the point of view of breeding structure is not the ratio of males to females in the colony on any one day, but the ratio of males to newly emerged females. This mating

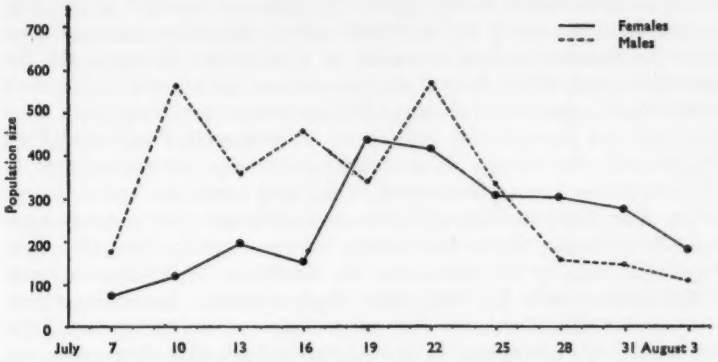

FIG. 4.—The daily population sizes at Cothill in 1950 have been averaged over short periods to minimise fluctuations resulting from sampling errors.

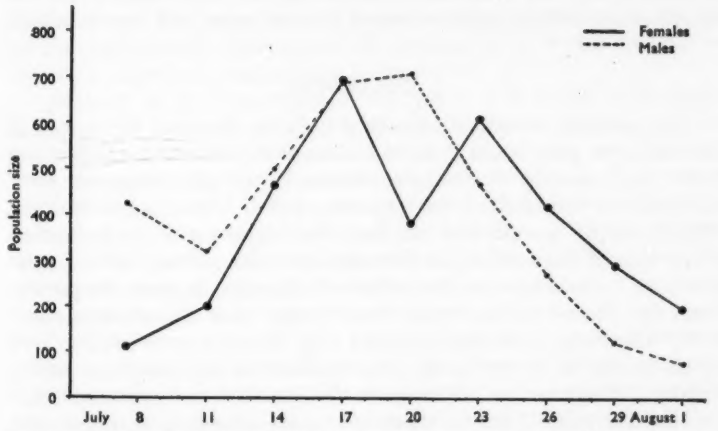

FIG. 5.—The diagram shows changes in population size in the Sheepstead Hurst colony during the 1950 season. There is a clear indication that males tend to emerge before the females.

system will reduce the effective population size by a small amount. However, this reduction will not be great, although its size cannot be estimated until it is known how much the ability of a male to copulate is impaired by previous matings. Selection in this type of breeding-community will be heavily in favour of any character which enables a male to find and mate with a female quickly.

It is unlikely that the early emergence of males is a function of the size of the pupa but is probably due to a complex physiological process which is subject to alterations through the agency of natural selection. The view that the timing of emergence of the two sexes is of a complex nature receives some support from the fact that in a brood of larvæ, which fed up rapidly in 1950, and emerged in October, instead of hibernating in the third instar, the males emerged later than the females instead of earlier as is normal. Selection for the accurate timing of the date of emergence must be heavy in *P. dominula* where the longest expectation of life, recorded since 1940, was 8·70 days and the shortest only 2·24 days. Dowdeswell, Fisher and Ford (1949) give the change in sex ratio and daily population size in *Maniola jurtina* L. on the Isles of Scilly, and again the same change in sex ratio from day to day is observed, although they seem to have started collecting about the middle of the season when the male population was at its maximum. It would be interesting to know if *Gonepteryx rhamni* L., and other single brooded butterflies which hibernate in the adult state and do not copulate till the spring, show the same early emergence of the males, and whether they come out of hibernation first. Moreover, it would be most interesting to make a comparison between species which hibernate as eggs, larvæ, or pupæ ; as adults, but with only one brood, as adults with more than one brood, and as adults with one brood in some areas and two in others.

6. DISCUSSION

The previous investigation into population size and the spread of the *medionigra* gene in an isolated colony of *P. dominula* by Fisher and Ford (1947) showed that the fluctuations in the gene frequency were too great to be ascribed to " genetic drift." On the other hand Wright (1948) has pointed out that the authors give no indication of the size of fluctuations in the selective value of the variant gene necessary to account for the observed changes in gene frequency. From the observed fluctuations he estimated that the selective value of the heterozygote *medionigra* would vary between semi-lethality and an advantage of 50 per cent. over *dominula* in the course of half a century. However, in arriving at this conclusion he assumes that the selective value " has no trend but varies according to non-secular fluctuations in conditions from year to year." On the contrary, the data for the years 1939 to 1950 give no indication that the relative selective advantages of the three genotypes has varied over this period. The disadvantage of the heterozygote over typical *dominula* was about 10 per cent. Between 1929 and 1939 the *medionigra* gene undoubtedly spread at a speed consistent with a selective advantage of about 20 per cent. An alternative hypothesis is that between 1929 and 1939 the population was reduced to an exceedingly small size on two or more occasions and that the gene increased in frequency

by " drift." However, Dr E. B. Ford informs me that he has written to many of the entomologists who collected *P. dominula*, at Cothill, between 1929 and 1939. At least one person collected each year and none record difficulty in obtaining a good " series " of moths on suitable days. The scarcity of moths would undoubtedly have been noticed if the seasonal population size had ever been reduced to a few hundred individuals. On the other hand, the populations of *Euphydryas aurinia* (Rott.) at Cothill and *P. dominula* at Tubney Wood have fluctuated in the past; a fact that has been recorded. The Tubney Wood colony was thought to be extinct, but actually the moths are still present at a very low density.

One other important suggestion made by Wright is that the effective and apparent population sizes may be very different. He suggests that if a female lays the majority of her eggs in one place, the offspring will be subjected to a similar environment and whole broods may either tend to all survive or all to be killed. Although it is agreed that experiments to determine the relationship between effective and apparent population size in many different types of breeding-community are essential, it must be pointed out that a large number of solitary insects, in fact, scatter their eggs over a wide area. The evidence obtained on the egg-laying habits of *P. dominula* both in the field and in the laboratory, as well as observations on the behaviour of the larvæ do not support the hypothesis that the fate of any individual is correlated with the fate of the brood through the agency of fortuitous fluctuations in the environment.

Because of the importance of this point, it is worth while reconsidering the evidence for it. Although Kettlewell (1942) considers that females lay a large proportion of their eggs in the area in which they themselves emerged from the pupa, the evidence of laboratory animals indicates that only a small proportion of the eggs are deposited in the first twenty-four hours after copulation. Moreover, field observations confirm this to some extent for females recaptured five, six, seven and eight days after they were first marked have frequently laid over a dozen fertile eggs during the hour or two between recapture and subsequent release. There is evidence from both Sheepstead Hurst and Cothill that females do, in fact, fly shortly after copulation. Lastly the eggs are not laid in batches, which adhere to a leaf or other object as in the majority of lepidoptera, but have no adhesive substance and are scattered freely over the vegetation. The type of habitat usually occupied by *P. dominula* is rough and much sub-divided by tussocks, reeds, bushes and trees so that even quite a small expansion of the area occupied by a brood, through the movement of the individuals in it, would ensure that it occupied a large number of microhabitats. The larvæ can move considerable distances and have been found as much as twenty feet up trees and they are also known to move from plant to plant. Because much of the elimination takes place in the late larval and pupal life, and because the larvæ scatter

from the food plant to pupate and are therefore less likely all to be influenced by the same micro-environment, it was decided to investigate the elimination of larvæ in the last instar. Twenty-two isolated Comfrey plants were selected at random from a much larger number of suitable plants and five larvæ which had just moulted into the last larval instar were placed on each. Isolated plants or plants in isolated clumps were used so that the possibility of larvæ emigrating or immigrating was minimised. A similar batch of larvæ was kept in the laboratory in order that the time of onset of pupation would be known, making certain that the results were not due to loss of larvæ by dispersal prior to pupation. The results of counts of larvæ on twenty-two plants on several occasions is given in table 34. The

TABLE 34

Number of plant	Number of larvæ still alive				Number of plant	Number of larvæ still alive			
	April 30	May 3	May 10	May 14		April 30	May 3	May 10	May 14
1	5	1	1	0	12	5	5	0	0
2	5	2	0	0	13	5	2	0	0
3	5	2	1	0	14	5	0	0	0
4	5	3	0	0	15	5	2	1	1
5	5	1	0	0	16	5	2	0	0
6	5	4	3	1	17	5	2	0	0
7	5	1	0	0	18	5	2	0	0
8	5	3	1	0	19	5	4	0	0
9	5	0	0	0	20	5	3	1	0
10	5	3	0	0	21	5	1	0	0
11	5	2	2	0	22	5	5	1	0
						110	50	11	2

data give no indication that there is a non-random elimination of larvæ with respect to plants. The experiment was not continued after 14th May because only two larvæ remained and on that date some larvæ in the laboratory started to pupate.

It is hoped to continue these experiments as well as to undertake others on the size of the larval population in the spring and the extent to which larvæ move from place to place, using genetically marked individuals. The two populations at Sheepstead Hurst and Cothill afford a unique opportunity not only for studying the changes in the selective value of certain genes, but also the breeding structure of populations and the factors controlling population size. At the moment it is possible to say that the general environment and the genetical composition of the two colonies is different although they are situated near one another. Also the changes in population size, which must be affected to a small extent by the number of eggs laid, and therefore the death-rate of adults, are not the same in the two localities.

It will be of interest in a few years time when the difference in the ecology of the two colonies is better known, to introduce the *medionigra* gene into the new colony and compare its behaviour in the two populations. Many inherited characters found in wild populations have been claimed as being of neutral survival value and the differences in their frequency in different populations has been attributed to " genetic drift " (*e.g.* Diver, 1940 ; Mayr, 1942). However, some of these factors as well as others which have been quoted as demonstrating " genetic drift " have since been shown to have considerable selective values (Dobzhansky, 1943, 1948 ; Carson and Stalker, 1949 ; Cain and Sheppard, 1950 ; and Sheppard, 1951). The apparent 20 per cent. selective advantage of the *medionigra* gene between 1929 and 1939 and its 10 per cent. disadvantage between 1939 and 1950, without any detectable sustained change in the environment, demonstrate very clearly how dangerous and misleading it is to claim that certain characters are neutral in survival value, even after quite extensive investigations.

7. SUMMARY

1. Two natural populations of the single-brooded moth *Panaxia dominula* L. (Arctiidæ), at Cothill and Sheepstead Hurst, Berkshire, have been investigated. Though situated only a mile apart, it was shown that their genetic compositions, as well as their environments, were different.

2. The Cothill colony had been under detailed observation since 1939. By means of marking the moths with cellulose paint, Fisher and Ford (1947) had determined the frequency of the *medionigra* gene from 1939 to 1946, and the population size from 1941 to 1946. The present paper extends these data to 1950.

3. It further shows that specimens heterozygous for the *medionigra* gene have been at a disadvantage of approximately 10 per cent. each year since 1939, though it is clear that they must have had a considerable selective advantage from 1929 to 1938.

4. Similar records for population-size are now provided for the second colony (at Sheepstead Hurst) for 1949 and 1950. *Medionigra* does not occur here.

5. A new variety of the moth was discovered in 1948. The form is genetically determined, but the exact nature of its control is still under investigation. Its frequency differs significantly in the two colonies.

6. A study of daily population sizes, and of the changes in sex-ratio, demonstrate that the males emerge earlier than the females.

7. There is no evidence that the marking of *P. dominula* alters its expectation of life, but there is some suggestion that it may affect the ease with which specimens can be caught (but see Note, p. 378.)

8. Fisher and Ford (1947) showed that the observed fluctuations in the frequency of the *medionigra* gene at Cothill must be controlled

by selection, and could not be attributed to "genetic drift" in the manner suggested by Sewall Wright. This has been further confirmed, and the criticisms made by Wright of the earlier part of this work are shown to be invalid (see Discussion).

Acknowledgments.—I am much indebted to Professor R. A. Fisher and Dr E. B. Ford for their kindness in encouraging me to make observations and collect data at Cothill for the continuance of the experiment which they started in 1939, and also for reading this paper in manuscript. Mr D. J. Finney, Mr J. M. Hammersley and Mr M. R. Sampford have assisted me with statistical advice and with some of the calculations. During part of the investigation I received a grant from the Department of Scientific and Industrial Research, for which grateful acknowledgment is made.

Note.—The data for 1951 make it unlikely that marking affects the case of recapture.

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THE USE OF SKIN GRAFTING TO DISTINGUISH BETWEEN MONOZYGOTIC AND DIZYGOTIC TWINS IN CATTLE

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1. INTRODUCTION

CATTLE twins have the same importance to geneticists as human twins, inasmuch as they make it possible to distinguish between inborn and acquired character differences by experiments of classical simplicity of design. Unfortunately, there is no completely reliable and objective method of distinguishing between monozygotic and dizygotic twins in cattle, and Dr H. P. Donald, who has discussed the problem in a recent issue of this journal (vol. 5, no. 1), therefore encouraged us to find out if the interchange of skin grafts between twins could serve such a purpose. The principle is obvious enough. It is an empirical fact that skin grafts do not long survive transplantation between mammals of ordinary genetic diversity (whether between unrelated individuals, siblings, or parents and offspring). "Homografts," as such grafts are called, should therefore fail when interchanged between

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dizygotic twins, which (according to a theory which will be shown to need some qualification when applied to cattle) are no more closely related genetically than ordinary siblings. On the other hand, it must be regarded as a genetical truism that grafts exchanged between monozygotic twins behave like autografts, *i.e.* grafts transplanted from one part to another of the same individual. Human clinical practice provides plenty of evidence that this is indeed the case, and skin grafting has already been used to clear up confusions of parentage (Franceschetti, Bamatter and Klein, 1948 ; McIndoe and Franceschetti, 1950). The fact that skin grafts may be interchanged with success among mice of highly inbred strains is, of course, a consequence of their becoming genetically more alike, but it has no practical bearing in the problem to be discussed here.

There is therefore a clear *prima facie* case for supposing that the interchange of skin grafts should make it possible to distinguish with complete certainty between monozygotic and dizygotic twins in cattle. We at first suspected that the problem of graft " dosage " might be a serious technical obstacle. The reaction against skin homografts becomes faster and more vigorous as the quantity of skin that is grafted increases, and the relative " dosage " of skin that can be grafted to cattle is obviously very much smaller than with a rabbit or mouse. These misgivings proved to be ill founded. Other things being equal, the intensity of the homograft reaction varies from one species to another, and in the cow it is of such violence as to make graft dosage a matter of altogether minor importance. In spite of this dispensation, and in spite of having overcome the purely technical difficulties of grafting, it proves that skin interchange *cannot* be used for distinguishing monozygotic from dizygotic twins in cattle. Dizygotic twins in cattle, whether of like or unlike sex, are in some manner mutually desensitised to grafts of each other's skin—a situation which clearly corresponds to the anomalous conformity of red cell agglutination types described in dizygotic cattle twins by Owen (1945) and Owen, Davis and Morgan (1946).

In section 4 of this paper, we shall describe the behaviour and fate of grafts exchanged (i) between unrelated cattle ; (ii) between twins classified by experienced observers as monozygotic ; (iii) between twins likewise classified as dizygotic ; (iv) between full siblings of separate birth ; and (v) between twins of different sex, and thus beyond question of dizygotic origin. We improved our operative methods while the work was in progress, and the majority of tests made use of a technique which we regard as very satisfactory—the transplantation of a number of individual discs of skin (" pinch " grafts) from the dorsum of the ear to the skin of the dorso-lateral aspect of the thorax—the "withers." The grafts were thus permanently " labelled " (see below) by having (and of course retaining, if they survived) the distinctive properties of ear skin. Wherever the colour patterns of donors and hosts allowed it, they were labelled by intrinsic

colour differences as well—*e.g.* by grafting pigmented skin to non-pigmented, or differently pigmented, recipient areas. On a number of occasions animals were repeatedly grafted from the same donor; and most animals received autografts as well as homografts to provide some estimate of the efficacy of healing uncomplicated by immunological reactions.

2. A NOTE ON THE SKIN HOMOGRAFT REACTION*

Skin homografts are destroyed by a mechanism that is known to belong to the general category of actively acquired immune reactions. Their initial healing is as strong as with autografts; they are as quickly and as richly vascularised, and they show the same outburst of epithelial proliferation that follows the establishment of a blood supply; and, in general, they behave just like autografts until the reaction they elicit from their host begins to overtake them. Just how soon this happens depends upon a variety of factors, of which the only two that are relevant here are (*a*) the quantity of foreign skin that is grafted: the higher the dosage, the shorter the survival time; and (*b*) the antigenic and therefore the genetic relationship between donor and recipient: the smaller the difference, the longer the time of survival. The second factor quantitatively outweighs the first.

When an animal has already received and reacted against homografts from the same donor, the sequence of events is modified. The second set of homografts is reacted against much more quickly; the vascular system of the grafts is soon disrupted, leaving them in an ischæmic condition; and epithelial proliferation is suppressed. This "immune" effect provides a valuable confirmatory test of incompatibility which we have repeatedly made use of.

The survival time of homografts exchanged between ordinary siblings or unrelated cattle has in our experience proved to be in the neighbourhood of ten days. (More exact information will be given below.) The reaction that brings about such rapid breakdown is "acute," and it is associated with an inflammatory reaction of extreme violence. "Acute" breakdown may be watched and diagnosed with certainty by naked eye inspection. The graft swells, because it is inflated with œdematous fluid and with frank hæmorrhages, and changes colour from the delicate pink associated with ordinary vascularisation to various shades of dirty yellow, brown or black. Meanwhile it hardens, so that instead of being resilient it moves as a whole when palpated; its rim becomes free from the surrounding host tissue, to which it had earlier united; the surface epithelium weakens and becomes wet and pasty, instead of firm, shiny and dry, and may be stripped off to reveal the discoloured and pitted surface of the dermis below. In due course the graft simply becomes a dry

* See Medawar (1944, 1945, 1946*a*).

and necrotic scab, which is undermined by the ingrowth of the host's own epithelium so that it eventually drops away.* Histological examination of homografts in the period that precedes final breakdown reveals all the phenomena of acute inflammation. Lymphocytes are the dominant inflammatory cell; the presence of granulocytes in any abundance is a consequence of secondary infection.

We shall have occasion in the text to describe a much milder, chronic breakdown reaction, the progress and completion of which cannot always be followed without error by mere naked eye inspection. Weak chronic reactions have been found to occur in grafts interchanged between certain dizygotic twins.

It is proper to say that other variables which govern the survival time of homografts and the quality of the reaction elicited by them have been eliminated in our experiments. The species factor is constant; the grafts are all orthotopic (*i.e.* are grafts of skin into positions formerly occupied by skin), so that peculiarities of blood supply or lymphatic drainage are irrelevant; and any physiological stress caused by operation, such as might prolong the survival time of homografts by causing the liberation of cortisone-like adrenal steroids (Billingham, Krohn and Medawar, 1951), is manifestly of trivial importance.

3. METHODS

Over most of the body surface bovine skin (plate 1, fig. 1) consists of three major layers: (*a*) a fully stratified superficial epidermis (plate 1, fig. 2) and its derivatives, the hairs, the bases of which penetrate deeply into (*b*) a very thick, tough dermis which is firmly united to (*c*) a layer of striped muscle, the "panniculus carnosus." Between this layer of muscle and the overlying dermis run the principal blood vessels and lymphatics of the skin. The epidermis of skin bearing pigmented hairs is itself usually pigmented.

The skin of the dorsum of the ear (plate 1, fig. 3) differs from that of the general body surface in that it bears a sparser hair crop, its dermis is thinner and composed of finer collagen fibres, and it is rather loosely united to the fascial layer that overlies the ear cartilage, a panniculus carnosus being absent.

After a few preliminary trials in which grafts were cut from the exceedingly thick tough skin of the dorso-lumbar region, this source was abandoned for the thinner and more mobile skin of the dorsum of the ears, from which it was possible to cut thinner grafts. A general description of the technique of experimental skin grafting is given by Billingham and Medawar (1951).

* The growth of native epithelium over the collagenous remnant of a broken-down homograft forms a scar that may be quite easily mistaken for a surviving ear skin homograft, if regular inspections have not been carried out. Three criteria distinguish such a scar from a genuine ear skin graft: it is always bald; its surface is smooth and shiny, instead of finely reticulate and matt; and it is rounded and raised up, whereas ear skin grafts are flat and, because of their thinness, recessed below the plane of the skin surface.

(i) *Preparation of the donor area*

After a preliminary close-clipping, shaving and thorough scrubbing with soap and water the dorsum of the ear was rinsed with a 0·1 per cent. solution of " Cetavlon" in 70 per cent. spirit which was allowed to dry on. Local anæsthesia was then induced by the subcutaneous injection of a total of 5-10 c.c. of a 2 per cent. solution of ethocaine hydrochloride (such as " Novutox ") at several sites below the surface of the prepared skin.

A number of separate " pinch " grafts were cut from the donor area so prepared—each a disc of skin, 10-12 mm. in diameter and comprising the epidermis and the full thickness of the dermis. To cut each graft a small tent of skin was raised with the aid of fine, sharp forceps which had been so adjusted that their points could be brought together with a pincer-like action, and the base of the tent was then cut through by horizontal incisions with a No. 12 scalpel. Even in young animals as many as 24 such grafts could easily be cut from the dorsum of one ear. Immediately after cutting the grafts were placed raw side down in a sterile Petri dish on the bottom of which had been placed a piece of filter paper soaked in Ringer's solution or normal saline. The donor area required no special treatment apart from a thick dusting with a sulphonamide powder. A protective scab rapidly formed over each graft donor site and this normally came off after about ten days, when the raw area had been fully resurfaced by epithelial ingrowth from the margins of the lesions.

(ii) *The recipient area*

For success a skin graft must be transplanted to a vascular bed and held in place under moderate perpendicular pressure until primary healing is complete. In our earlier experiments, grafts were transplanted to the outer aspect of the forelimb above the " knee " (plate 1, figs. 4, 5, 6) and held in place by bandages and a plaster casing. Besides being extremely awkward for the surgeon, this site proved to be unreliable, for the plaster sheath sometimes slipped and several of the grafts were consequently lost. It was therefore abandoned for one located fairly high dorso-laterally on the thorax, just posterior to the scapula (*i.e.* on the side of the " withers ") (plate 1, figs. 7, 8, 9). The hair over a fairly extensive rectangular area was close-clipped and the central region, where the actual graft beds were to be prepared, was shaved and anæsthesia induced by infiltration with 10-15 c.c. of local anæsthetic. A separate bed to receive each graft was then cut, exactly as one cuts a " pinch " graft, except that the graft itself is discarded, only its bed being required. Graft beds prepared in this way increase in depth from the margin to the centre, where they extend down to the vascular fascial planes overlying the panniculus carnosus. Some care was taken to make the beds of such a shape and size that the grafts fitted into them exactly.

After removing all free blood from the graft beds with sterile gauze swabs, the grafts were pressed firmly into position. After the transplantation of the grafts a sheet of home-made tulle gras (a self-adherent dressing prepared by impregnating fine open-wove bandage with vaseline) was pressed smoothly over the operation field so as to cover the grafts completely and leave a marginal overlap of 1-2 cms. Immediately over the tulle was placed a pressure pad of approximately the same size and comprising several thicknesses of sterile gauze. To prevent these dressings from being displaced the area of clipped skin round the operation field was painted with a latex solution to make it sticky, and several lengths of 4-inch wide bandage were then pressed firmly into such a position that they intersected at right angles across the immediate graft dressings, thereby preventing side-slip. Finally, to secure adequate vertical pressure, a large square pad of cotton wool, about 12×12 inches, was placed over the entire field and held in this position under moderate compression by means of a hessian truss which was passed right round entire thorax, the free ends being laced together ventrally (plate 1, fig. 8). This technique has given complete success in animals ranging in age from a few weeks to a year or more. As many as 28 grafts have been transplanted to this site and there is no reason to suppose that it would not accommodate many more if required.

Primary inspection was carried out seven to fourteen days after operation. Owing to the powerfully adhesive properties of the latex no attempt was made to remove all the dressings at this stage. An inspection window was cut over the actual graft field and after inspection the young grafts were protected from the animal's attempts to lick them by covering them with a sheet of gauze which was secured to the old dressings with latex solution. This secondary dressing was normally removed after five to seven days, no other being necessary.

(iii) *Skin autografts*

As mentioned above, pigmented ear skin—nearly always red or black in the animals available—was whenever possible transplanted to recipient areas cut in white skin. The fate of skin autografts was studied in some detail to provide a standard against which to compare the fate of homografts. In almost all our experiments a group of autografts was transplanted simultaneously to the same operation field to act as "controls." As was to be expected, these autografts invariably "took" provided that the dressings remained in place during the critical period of primary healing, which lasts three or four days. By the first inspection, normally carried out eight to twelve days post-operatively, the grafts had healed in completely and their margins, though visibly distinct, were firmly united to the adjacent host skin. From the surface of the grafts a dry, scaly cuticle—the "ghost graft"—could be cleanly peeled off to reveal a dry,

healthy-looking epithelium which was normally slightly pinkish in colour. In grafts cut from deeply pigmented skin it was observed that the pigmentation of the grafts underwent a considerable though transient dilution, which reached its peak by about the 30th day when the centres of the grafts were often completely depigmented. Recovery of pigmentation was often not complete until about the 50th day. The more peripheral regions of the grafts rarely manifested this severe bleach-out, and from about the 20th day the normal depth of colour returned progressively from this marginal region inwards.

With pigmented grafts transplanted to white skin it was observed that the pigmentation of the graft encroached progressively upon the surrounding white host skin from about the 30th day onwards, so that after 100 days a graft had become surrounded by an annulus of pigment spread 1 cm. or more in width (plate 1, figs. 4, 5, 6). This spread of pigmentation did not affect the hairs in the white skin into which it encroached (plate 1, fig. 5), and the hairs remained white. The process is exactly analagous to that which is known to occur in the guinea-pig (Billingham and Medawar, 1948, 1950).

The hair follicles carried over with the grafts undergo a phase of cystic hyperplasia during which the remains of the old hair shafts are shed. All the hairs that later grow from the grafts are from follicles of new formation. New hairs do not begin to pierce the graft surface until about the 40th day.

As a rule the grafts remained permanently recessed, lying slightly below the general level of the host skin. This is undoubtedly due to the fact that the dermis of ear skin is very much thinner than that of body skin.

4. EXPERIMENTAL RESULTS

(I) *The exchange of skin homografts between unrelated cattle*

As a preliminary to attempting to differentiate between mono- and dizygotic twins, it was of course necessary to confirm that skin homografts exchanged between unrelated cattle of the same or, of course, different breeds elicit and succumb to a homograft reaction of appreciable intensity.

Skin homografts were reciprocally exchanged (*a*) between six pairs of unrelated animals which were individually of twin birth; and (*b*) between four pedigree Ayrshire cattle, each of separate birth (*i.e.* not members of twin pairs), in such a manner that each animal received homografts from each of the other three (see fig. 1). In all animals the results were perfectly clear-cut and consistent. Although there was ample evidence that these grafts had at first healed soundly, they rapidly became swollen, œdematous and severely inflamed. The graft surface became weak and pasty so that it could easily be picked off with fine forceps to reveal a grossly hæmorrhagic dermis. The rims or margins of the grafts became free and as the graft became

dry, discoloured and necrotic, it was progressively undermined by ingrowth of native epithelium from the margins. Finally the ingrowing sheet of native epithelium cut through the slender central stalk of attachment of the scab-like graft residue, which then dropped off. Histologically this " acute " homograft reaction was accompanied by round cell infiltration of the dermis and even the epidermis of the graft. In no animal did the epithelium of a homograft survive beyond the 15th day and in the majority breakdown was complete by the 9th day.

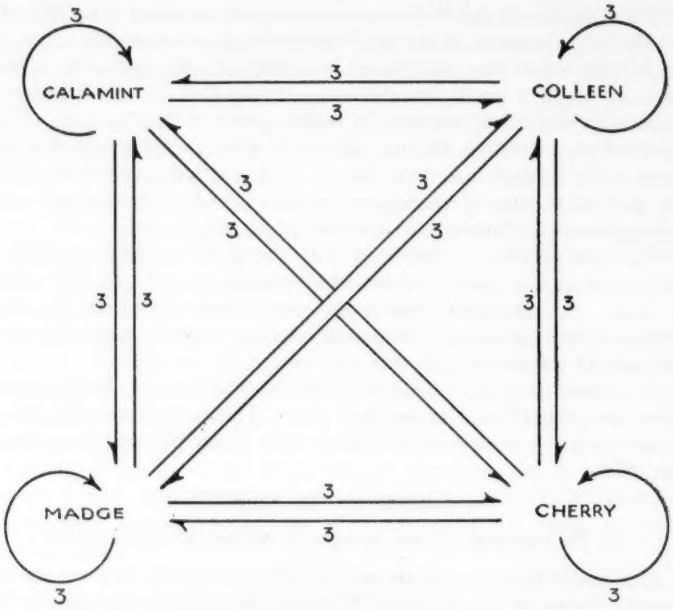


FIG. 1.—Illustrating the skin transplantation experiment carried out between four pedigree Ayrshire cattle, each of separate birth. Each animal received three homografts from each of the others and three autografts as well.

Although it was not possible to time accurately the completion of breakdown in all homografts, sufficient data were obtained to indicate that it did indeed vary within the stated limits. Moreover, no detailed information was gained concerning the effect of graft dosage. It is, however, interesting to note that, with several animals to which only a single homograft was transplanted, breakdown was nevertheless complete by the 9th day. It is scarcely necessary to state that in the few animals to which a second homograft was transplanted from the original donor after the breakdown of a first, the second graft broke down as quickly as the first, though no attempt was made to find out if it broke down more quickly.

These experiments made it clear that skin homografting gives rapid and conspicuous evidence of the genetic disparity between unrelated cattle of the same breed.

TABLE 1

Number *	Sire	Dam	At operation		Type of operation	Observation period (days)	Survival time of homografts (days)
			Age (days)	Wt. (lbs.)			
DZ 10A	Friesian	Shorthorn	92	184	Autograft and exchange homograft	153	<14
HZ 9A	Friesian	Shorthorn × Friesian	62	164			<14
DZ 10B	Friesian	Shorthorn	127	227	Autograft and exchange homograft	118	<11
DZ 11B	Friesian	Ayrshire	125	192			<11
DZ 11A	Friesian	Ayrshire	136	208	Autograft and exchange homograft	107	<11
HZ 9B	Friesian	Shorthorn × Friesian	108	218			<11
MZ 12A	Friesian	Friesian cross	126	110	Autograft and exchange homograft	107	Grafts lost †
HZ 14B	Ayrshire	Ayrshire	14	78			<11
CALAMINT	Ayrshire	Ayrshire	273	570	Autografts and exchange homografts ‡	9	< 9
COLLEEN	Ayrshire	Ayrshire	318	520			
CHERRY	Ayrshire	Ayrshire	89	220			
MADGE	Ayrshire	Ayrshire	77	190			
BH 3A (♀) BH 3B (♂)	Shorthorn	Friesian	29	100	Autografts and *inter-pair* homografts §	84	Homografts *between* pairs survived <15 days, section 4 (v).
BH 4A (♀) BH 4B (♂)	Ayrshire	Ayrshire	27	80		Died	...

* All animals except BH 3B and BH 4B were females.
† As a result of plaster slip in the foreleg position.
‡ See fig. 1 and description in text, section 4 (i).
§ See fig. 3 and description in text, section 4 (v).

(ii) *The exchange of homografts between allegedly monozygotic twins*

The provisional diagnosis of the monozygotic or dizygotic origin of the twins used in the experiments to be described in this section and the next was, of necessity, based on orthodox phenotypic appraisal.

Skin grafts were reciprocally exchanged in various dosages—usually not less than 6 standard pinch homografts per animal—between the two members of each of 10 separate and unrelated pairs of allegedly monozygotic twins, all except one pair being females. In every pair except one, the grafts behaved just like " control " autografts throughout the period of observation.

The one exceptional pair, MZ 1A and MZ 1B, operated on when 37 days old, became sufficiently unlike as they grew up to make us question their original classification as monozygotic. By this time single (pigmented) homografts exchanged between them had remained perfectly normal to outward appearance for 560 days, and each was surrounded by a wide band of black pigment spread. A confirmatory operation was nevertheless undertaken, and 24 ear skin grafts were cut from each member of the pair and transplanted, with 4 autograft controls, to the other.

When the second stage grafts were of 23 days' standing, the homografts (which should in theory have been indistinguishable from the autografts) were seen in both animals to be slightly inflamed. At the same time,

the first-stage homografts on both animals—now 584 days old—became much paler in colour, and the surrounding ring of pigment spread became light brown or almost straw coloured. The inflammation and the pigment dilution both subsided within the next 27 days, and the grafts became to outward appearance perfectly normal.

TABLE 2

Number *	Sire	Dam	At operation		Type of operation		Observation period (days)	Notes †
			Age (days)	Wt. (lbs)	Homografts interchanged	Autografts		
MZ 1A MZ 1B	Friesian	Friesian	37	138 143	1	...	630	...
MZ 1A MZ 1B	Friesian	Friesian	597	884 982	24	4	69	Second stage operation: see text, section 4 (ii)
M 3A M 3B	Friesian	Friesian cross	148	246 195	4	...	100	Two calves from a set of bull quadruplets
MZ 8A MZ 8B	Shorthorn	Shorthorn	169	202 207	4	...	140	...
MZ 9A MZ 9B	Shorthorn	Guernsey	544	802 756	6	6	43	Animals also received inter-sib grafts: see section 4 (iv)
MZ 10A MZ 10B	Shorthorn	Shorthorn	182	248 250	6	...	82	...
MZ 12A MZ 12B	Friesian	Friesian cross	133	210 213	4	...	90	See also section 4 (i)
MZ 13A MZ 13B	Shorthorn	Shorthorn × Friesian	489	713 689	6	6	18	Animals also received inter-sib grafts: see section 4 (iv)
MZ 14A MZ 14B	Shorthorn	Shorthorn	116	146 142	4	...	90	...
MZ 16A MZ 16B	Welsh	Welsh	90	200 198	6	...	82	...

* All the animals were female except M 3A and B.
† Homografts survived in all animals.

In the light of the findings of the next section, these reactions gave weak evidence of an exceedingly feeble and transient homograft reaction, so confirming the doubt that had arisen about the justice of the original classification. It will be noticed, however, that the initiative for the re-examination of the diagnosis came from the evidence of ordinary phenotypic appraisal and not from the evidence of grafting.

(iii) *The exchange of homografts between allegedly dizygotic twins of similar sex*

In this group of experiments skin homografts were exchanged between the members of 7 different pairs of allegedly dizygotic female twins. (A further group of experiments involving dizygotic twins of unlike sex will be described in a later section.) The obvious dissimilarity between the two members of most of the pairs made the likelihood of faulty classification fairly remote.

In not a single animal did any of the homografts undergo the rapid, clear-cut breakdown that might have been anticipated from the results

obtained when grafts were exchanged between unrelated animals, *i.e.* between animals of unquestionable genetic disparity.

In all these pairs of twins, irrespective of whether there had at any stage been the slightest indication of a reaction after the transplantation of the first set of homografts, a second set of homografts was subsequently exchanged, and in many this was followed by a third set of homografts, making a total dosage of 20-30 homografts

TABLE 3

Number *	Sire	Dam	At operation						Total number of homografts interchanged	Observation period (days)	Notes †
			First stage		Second stage		Third stage				
			Age (days)	Wt. (lbs.)	Age (days)	Wt. (lbs.)	Age (days)	Wt. (lbs.)			
DZ 2A DZ 2B	Friesian	Ayrshire	25	87 93	575	750 806	...	...	25	630	Delayed breakdown: see text, section 4 (iii)
DZ 10A DZ 10B	Friesian	Shorthorn	224	407 369	466	780 708	...	...	18	327	See also section 4 (i)
DZ 11A DZ 11B	Friesian	Ayrshire	164	221 217	464	732 676	...	...	16	395	Transient reaction: see text, section 4 (iii), see also section 4 (i)
DZ 12A DZ 12B	Shorthorn	Shorthorn	83	159 165	403	643 699	—	—	28	380	...
DZ 14A DZ 14B	Shorthorn	Shorthorn	198	277 293	429	624 644	450	624 644	32	327	Transient reaction: see text, section 4 (iii)
DZ 15A DZ 15B	Ayrshire	Shorthorn	133	218 255	375	626 679	422	651 688	40	348	...
DZ 16A DZ 16B	Friesian	Friesian	80	140 116	322	494 468	343	494 468	33 on 16A 39 on 16B	338	...

* All animals female.

† Survival of homografts on all animals except DZ 2A (see text).

per animal. These subsequent transplantations were carried out in an attempt to increase the actual dosage of "foreign" skin born by the animals and thereby elicit or at least augment the reaction that the first set of grafts evidently failed to provoke.

These seven pairs of twins were observed at regular intervals over a considerable period of time (300-400 days). In four pairs there was not the slightest indication of a reaction—the grafts behaved like autografts in all respects, save of course with regard to their colour, which remained donor specific. In two pairs only was there evidence of a distinct though considerably delayed reaction; in a third pair there was no more than a suspicion of a reaction. These animals will now be described in further detail.

DZ 2A and 2B.—Single black homografts were interchanged and grafted to white areas. The homograft from 2B on 2A provoked a chronic low-grade and apparently cyclical (as opposed to progressive) inflammatory reaction, associated with scabbing and partial loss of its surface epithelium. The reaction was first recorded at a routine inspection on the 167th day,

and it culminated in apparent breakdown by the 204th day. The interpretation of the fate of the graft was complicated by the progressive development and persistence of a wide band of pigment spread into the white skin around it.

After 550 days a second set of 24 homografts was exchanged between the pair; and though the grafts appeared perfectly normal as late as the 34th day after grafting, they eventually elicited a reaction which culminated in total breakdown by the 60th day (plate 2, figs. 12, 13). The band of pigment spread around the original homograft was quite unaffected by the reaction—an apparent anomaly discussed in detail by Billingham and Medawar (1950), who have given evidence that such pigmentation is not due to the survival of foreign pigmentary cells.

The grafts from 2A on 2B showed absolutely no trace of a homograft reaction at any stage.

DZ 11A and 11B.—In this pair the story is essentially the same. In the first stage operation, four black ear skin homografts were interchanged, and in both animals they elicited a low-grade chronic reaction with persistent scaling and scabbing of the grafts and erosion of the surface epithelium. In this pair, as in DZ 2, the interpretation was complicated by the progressive development and persistence of pigment spread, but breakdown was almost certainly complete by the 69th day, if not earlier. The second stage operation was done 300 days after the first, and 12 homografts were interchanged. Breakdown was complete by the 36th day in both animals. Here, as before, the annuli of pigment spread from the original homografts was unaffected (plate 1, figs. 4, 9).

DZ 14A and 14B.—Four red ear skin homografts were interchanged at the first stage operation and remained perfectly normal to outward appearance throughout. Twenty-four red ear skin homografts were interchanged at the second stage operation 252 days later: these went through an inflammatory phase, more pronounced in 14B than in 14A, but thereafter settled down to normality.

In summary, the results of exchange transplantation on our seven pairs of dizygotic twins of similar sex may be classified thus: (*a*) four pairs in which the homografts remained perfectly normal throughout; (*b*) one pair in which a perceptible, but trivial and transitory homograft reaction was elicited; (*c*) two pairs in which a feeble, dilatory and long drawn out reaction eventually culminated in the destruction of the foreign skin.

We now turned to consider two hypotheses which, however improbable, might conceivably account for this anomalous behaviour:

(1) That the cattle were of a sufficiently high level of inbreeding to reduce the homograft reaction to one of trivial and, in most cases, imperceptible intensity. This possibility could clearly be checked by grafting between siblings of the same breeds but of *separate* birth, as described in section (iv). The *prima facie* case for this hypothesis was clearly weakened by the violence of the homograft reaction between cattle of the same breed though not of close familial relationship, together with the prolonged inbreeding known to be required

by mice before the skin homograft reaction is reduced to an intensity which allows the prolonged or indefinite survival of foreign skin.

(2) That the majority of the twins classified as dizygotic were in fact monozygotic. To refute this hypothesis, grafts were interchanged between dizygotic twins of dissimilar sex. Had we not started with the reasonable presumption that homografts on cows would behave in a manner analagous to homografts on the common laboratory mammals, these tests would of course have been made at a much earlier stage. They are described in section (v).

(iv) *Exchange transplantations between full siblings of separate birth*

The experiments to be described in this section comprise two independent tests of exactly similar design. In each test we made use of two (allegedly) monozygotic * female twins and a younger full sister ; the group of three was so operated upon that each animal received six homografts from each of its sisters and six autografts from itself (fig. 2).

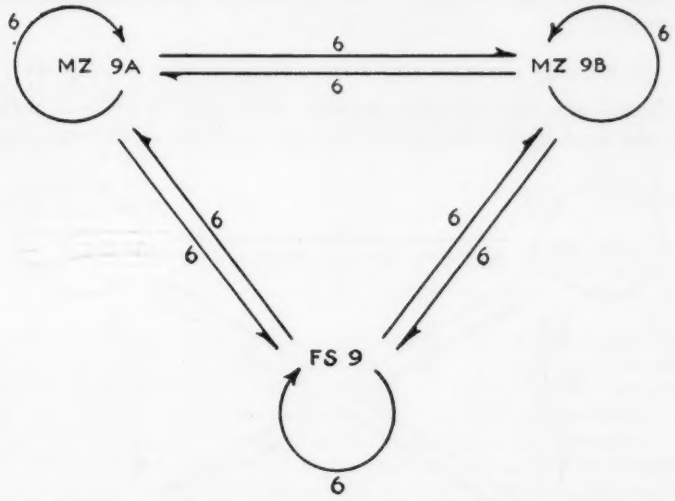


FIG. 2.—To illustrate the transplantations carried out between full siblings of separate birth. Animals MZ 9A and B were classified as monozygotic female twins, while FS 9 was a younger full sister. Each animal received 6 homografts from each of its sisters and six autografts from itself.

The results of both groups of tests were incisively sharp. The six homografts which the younger full sister received from each of the grown-up twins had become completely necrotic by the 15th day. The six homografts transplanted from the younger sister to each of the grown up twins broke down more quickly still. The autografts,

* Dizygotic twins would clearly have been preferable, but they were not available.

and the grafts exchanged between the twins, remained perfectly normal throughout. The violence of the homograft reaction is made clear by plate 2, figs. 10, 11.

TABLE 4

No.	Sire	Dam	Age (days)	Wt. (lbs.)	Observation period (days)
MZ 9A MZ 9B FS 9	Shorthorn	Guernsey	539 175	802 756 200	43
MZ 13A MZ 13B FS 13	Shorthorn	Shorthorn × Friesian	489 27	713 689 110	18

For the plan and results of the experiment, see fig. 2 and text above.
See also section 4 (ii) for pairs MZ 9 and MZ 13.

It was concluded that siblings of separate birth are in no wise exempt from the ordinary consequences of skin transplantation immunity.

(v) *Exchange transplantations between dizygotic twins of unlike sex*

Grafts were interchanged between three pairs of twins of unlike sex and kept under observation for not less than seventy-one days.

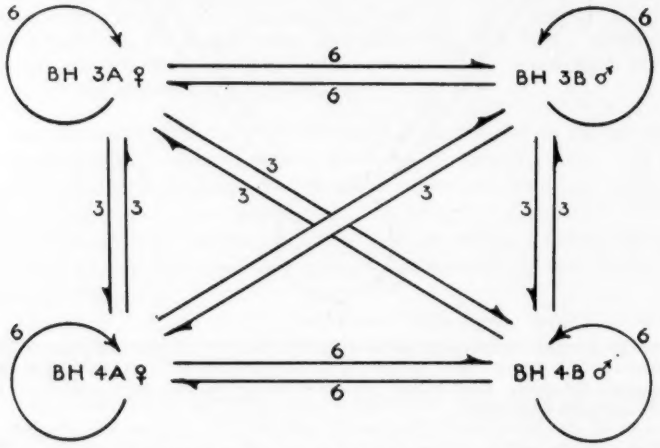


FIG. 3—Illustrating the transplantations carried out between two pairs of dizygotic twins of opposite sex so that each animal received six autografts, six homografts from the other member of its pair, and three homografts from each member of the other pair of twins.

The results prove without ambiguity that dizygotic twins of unlike sex are completely tolerant to grafts of each other's skin.

BH 1A (male: red roan) and BH 1B (female: blue roan *i.e.* with some mixture of white hairs with the black).—Each member of the pair received 6 autografts and 6 homografts from the other member of the pair. The female was destroyed on the 71st day after operation, and dissection of the reproductive system confirmed that it was a freemartin. Its homografts remained perfectly normal—*i.e.* autograftlike—throughout, both microscopically and to outward appearance (plate 2, figs. 18, 19). The homografts on BH 1A were observed for 124 days. A biopsy specimen taken at the 41st day gave histological evidence of a very weak homograft reaction in the form of circumscribed perivascular clusters of round cells (plate 2, figs. 16, 17), but the reaction did not progress and the grafts returned to normal.

BH 3A (female: black and white) and BH 3B (male: blue roan); BH 4A (female: red and white) and BH 4B (male: red and white).

TABLE 5

No.	Sire	Dam	At operation		Observation period (days)	Notes *
			Age (days)	Wt. (lbs.) (approx.)		
BH 1A (M) BH 1B (F)	Shorthorn	Shorthorn × Friesian	55	170	124 71	Freemartin
BH 3A (F) BH 3B (M)	Shorthorn	Friesian	29	100	109	Freemartin
BH 4A (F) BH 4B (M)	Ayrshire	Ayrshire	27	80	109 Died	Freemartin

The plan and results of the operations are described in the text and shown in fig. 3.

* See also section 4 (i) for pairs BH 1 and BH 2.

These two pairs of animals were operated upon in a single group: each animal received 6 autografts, 6 homografts from the other member of its pair, and three homografts from each of the two members of the other pair (fig. 3). BH 4B unfortunately died within 24 hours of operation; the other three received their last inspection 109 days after operation. All the homografts exchanged *between* the members of a twin pair remained to outward appearance perfectly normal throughout (plate 2, figs. 14, 15). The homografts transplanted between individuals of different pairs all broke down by the 15th day, though not simultaneously (see Discussion p. 394.)

Both females proved on autopsy to be freemartins.

These results exactly confirm those found by exchanging grafts between dizygotic twins of like sex and so make it unnecessary to enquire further into the possibility that all the latter were in reality monozygotic.

5. DISCUSSION

(i) *The use of skin grafting for twin diagnosis in cattle*

The practical import of the work described above is that graft interchange cannot be used as a method for distinguishing between twins of monozygotic and dizygotic origin in cattle. Experiments are now to be begun that make use of a different principle : the grafting of skin from both members of a twin pair to a third animal unrelated to either. As a further refinement, the recipient might be immunised beforehand by grafts from one only of its two donors. Even small differences between the breakdown times of the grafts and the severity of the histological reactions they elicit would give evidence of a genetic disparity between the two donors ; for it is already known (*a*) that orthotopic skin grafts of a genetically uniform population behave identically, even in the finest histological detail, when grafted simultaneously to a single recipient (Medawar, 1945), and (*b*) that grafts transplanted from a variety of donors to a single recipient differ very widely in their survival times and in the violence of the reactions they provoke (Medawar, 1945 ; *cf.* also Billingham and Medawar, 1950).

Preliminary results from the interchange of grafts between pairs BH 3 and BH 4 (see section v) show that the method described in this section is worth further investigation.

(ii) *The cause of the anomalous behaviour of grafts exchanged between dizygotic twins*

The bare fact revealed by the experiments described in this paper is that skin homografts interchanged between dizygotic twins are in a high degree, though not always, mutually acceptable. To say that dizygotic twins are mutually " desensitised " to grafts of each other's skin is to hint at one possible explanation of this anomaly. In its technical sense, " desensitisation " refers to an acquired indifference to an otherwise active antigen that may be achieved by prolonged exposure to the antigen in very low, or initially very low, doses. It is not yet known whether or not minute ascending doses of tissue homografts can confer tolerance upon their recipients, but there is clear evidence that a naturally-occurring process analagous to desensitisation occurs in dizygotic bovine twins. Lillie's (1916, 1917) endocrinological interpretation of the " freemartin " condition in the female member of dizygotic twins of unlike sex (*cf.* also Tandler and Keller, 1911) turns upon the fact that bovine twins, normally synchorial, actually establish a vascular anastomosis during uterine life. It is also a fact that the majority of dizygotic twins have identical red cell antigens (Owen, 1945 ; Owen, Davis and Morgan, 1946), although upwards of 40 red cell antigens are known in cattle (Ferguson, 1940 ; Ferguson, Stormont and Irwin, 1942 ; Stormont and Cumley, 1943) and uniformity of red cell antigens between sibs other than twins is

rare. Owen has produced evidence that the blood of dizygotic twin cattle may contain a mixture of red cells of different antigenic type ; since red cells do not reproduce and have short lives, he infers that red cell precursors are interchanged between the twins *in utero* and survive thereafter as cellular homografts. The dizygotic twin calf at birth is already, in fact, a genetical chimæra.* The experiments described in this paper provide direct confirmation of Owen's hypothesis.

In one important respect the mechanism of acquired tolerance to tissue homografts in twin cattle differs from desensitisation in the ordinary sense. The embryo is immunologically inactive : it does not manufacture antibodies and cannot therefore be said to become " desensitised " to an antigen to which it is not in any case sensitive. It may be that a desensitisation analagous to that conferred by minute increasing doses of an allergen occurs when, in fœtal life, an antigen confronts the embryo's gradually awakening faculty of immunological response. There may also be the more profound theoretical reasons adduced by Burnet and Fenner (1949) for supposing that animals become unresponsive to potential antigens to which they are exposed as embryos. The whole problem of embryonic adjustment and response to antigens is to receive a fuller analysis than is made possible by experiments on cattle. In the meantime, it will be noticed that our own experimental results relate to young cattle only : we have no evidence of the behaviour of homografts on cattle fully grown.

Dizygotic twin pairs are not always completely tolerant to grafts of each other's skin, and chronic breakdown reactions of delayed onset have been described in section (iii). The reason may be the same as that adduced by Chapin (1917) to account for variations in the degree of sexual abnormality to be found in freemartins ; *i.e.* that it is due to differences between the times in fœtal life at which vascular anastomosis is established, and possibly between the degrees of anastomosis achieved.

One possible, but very specialised use of graft interchange methods may be mentioned. If our interpretation—in all essentials Owen's—is correct, then dizygotic twins that do *not* establish a vascular anastomosis *in utero* should not be tolerant to grafts of each other's skin. The female member of a pair of unlike sex should, moreover, not be a freemartin. It follows that exchange transplantation should be able to discriminate at a very early age between freemartins and sexually normal females, though it may well prove that blood typing methods (Owen, 1945) are better. A second possibility of much wider import is the field of research that is now shown to be open

* In the current embryological (which is also the classical) sense, a " chimaera " is an organism whose cells derive from two or more distinct zygote lineages, and this is the sense which the term " genetical chimaera " is here intended to convey. " Genetical mosaic " is less suitable, because a mosaic is formed of the cells of a single zygote lineage. In a sense, the dizygotic cattle twin is what the botanist would call a " graft hybrid."

to the grafting of tissues between animals of very different genetical constitutions. We are proposing to study the possible desensitisation of mouse embryos by inoculated foreign cells with this object specially in mind.

It may seem surprising that the interchange of red cell precursors should confer tolerance upon homografts of, effectively, skin epithelium; but it should be pointed out (*a*) that there is already known to be some degree of overlap between the antigens of leucocytes and of skin epithelium (Medawar, 1946*b*), and (*b*) that red-cell precursors are not likely to be the only cells that are interchanged. Leucocyte precursors are certain to be among them. The fact that the dizygotic twin cow is a genetical chimæra—in particular, that the freemartin contains genetically male cells—may make it necessary to qualify and enlarge the purely humoral interpretation of the freemartin condition : its shortcomings are now well known (Moore, 1947).

The peculiar violence of the homograft reaction in cows still requires an explanation.

In the light of our findings, it is reasonable to suppose that the apparently anomalous survival into the adult life of birds of cellular or even organ homografts transplanted into embryos (*cf.* the review by Rawles, 1948) will prove to have the same explanation as the tolerance of dizygotic twin cows to grafts of each other's skin—except that, in birds, the inoculation of embryos with foreign embryonic cells is brought about by experimental artifice and not by natural means.

6. SUMMARY

An investigation has been made of the value of skin grafting for discriminating between monozygotic and dizygotic cattle twins, in accordance with the general rule that skin grafts exchanged between genetically dissimilar mammals are rapidly destroyed.

Skin was accordingly interchanged between cattle of the following degrees of affinity :—

(1) Unrelated animals, individually of twin birth ; animals related only by being of the same pedigree breed, and individually of separate birth.

(2) Full siblings of separate birth.

(3) Dizygotic twins of unlike sex.

(4) Twins of similar sex classified by ordinary phenotypic appraisal as dizygotic.

(5) Twins classified by ordinary phenotypic appraisal as monozygotic.

Both monozygotic and dizygotic twins are tolerant to grafts of each other's skin. Skin grafts interchanged between animals of the other degrees of affinity are very rapidly destroyed.

Not all dizygotic twin pairs are completely tolerant to grafts of

Plate I

FIG. 1.—Vertical section through the full thickness of cow's skin from the withers area to illustrate its general anatomy (see text). See fig. 2 and contrast fig. 3. ×20.

FIG. 2.—A view in higher power of the preparation illustrated by fig. 1. The superficial epidermis is rather thinner and more irregular than that of ear skin (fig. 3). ×70.

FIG. 3.—Vertical section through the full thickness of the skin of the dorsum of the cow's ear. Contrast fig. 2. ×70.

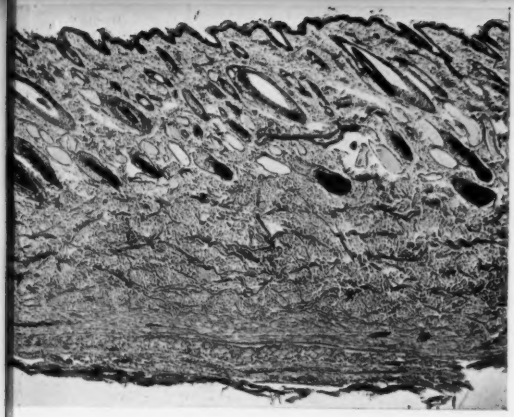
FIG. 4.—Recipient: DZ 11A. Showing 4 homografts of 69 days' standing, and one control autograft, transplanted to the outer aspect of the foreleg. Although all grafts are surrounded by rings of "pigment spread," the homografts are depigmented, scabbed and inflamed (see text).

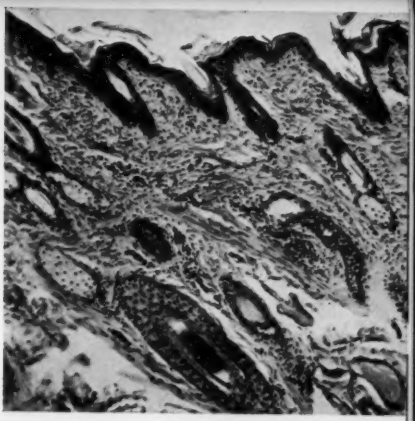
FIGS. 5 and 6.—Recipient: HZ 9A. A single autograft of 153 days' standing photographed before (fig. 5) and after (fig. 6) shaving, to show that the white hairs of the operation field are unaffected by the pigment spreading from the graft centre, which is clearly seen in fig. 5.

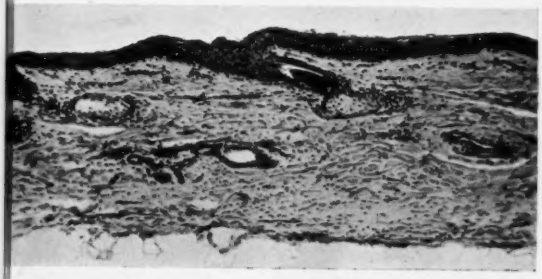
FIG. 8.—Illustrating the hessian truss that is used to maintain a perpendicular pressure upon grafts transplanted to the withers region (*cf.* figs. 7 and 9).

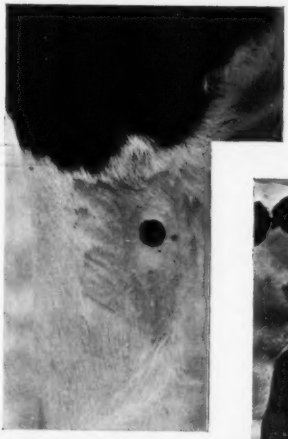
FIG. 7.—Recipient: DZ 16B. To illustrate the now preferred operation field in the wither area: a group of 24 "third-set" pigmented homografts from DZ 16A, here shown 38 days after transplantation to an area of white skin.

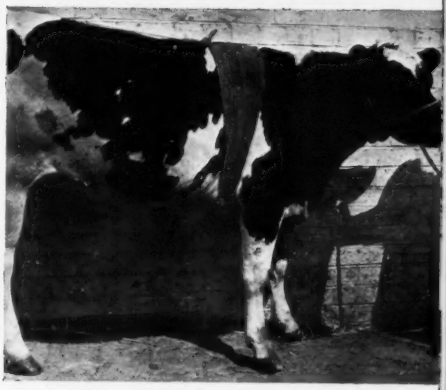
FIG. 9.—Recipient: DZ 11A. Photograph taken 36 days after transplanting a second set of 12 pigmented ear skin homografts from DZ 11B. Pigment spread is in progress, but the grafts themselves are grossly inflamed and scabbed (see text).

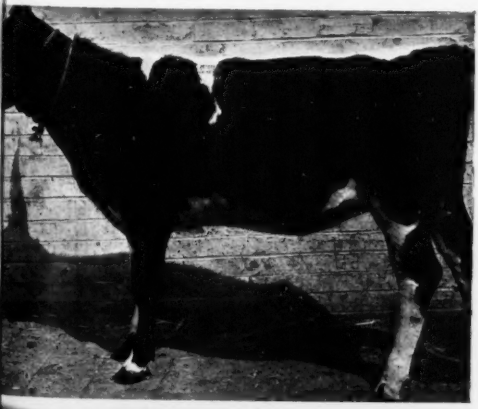












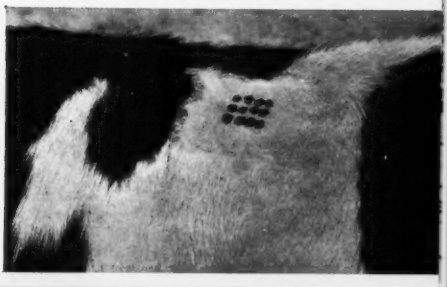


FIG. 10.—Recipient: MZ 13B. Transverse section through a homograft 14 days after transplantation from a younger full sister, MZ 13S, showing complete necrosis of the graft, which has been riddled by round cells and now forms a dried "mushroom" scab in the process of being undermined by native epithelium. See also fig. 11. ×18.

FIG. 11.—Recipient: MZ 13S. Transverse section through a homograft 14 days after transplantation from an older full sister, MZ 13B. The homograft reaction is far advanced, but not quite complete. The dermal papillæ have been densely infiltrated with round cells and the basal layer of the epidermis is already disintegrating. See also fig. 10. ×120.

FIGS. 12 and 13.—Recipient: DZ 2A. Transverse section through the edge of one of a second set of homografts 60 days after transplantation from DZ 2B, illustrating a violent reaction of delayed onset. The graft is now totally necrotic: notice the incursion of native skin epithelium on either side, and the still active round cell reaction in the dermis. Fig. 12 ×56, fig. 13 ×100.

FIGS. 14 and 15.—Recipients: BH 3B and BH 3A, showing autografts and reciprocally exchanged homografts of 109 days' standing.

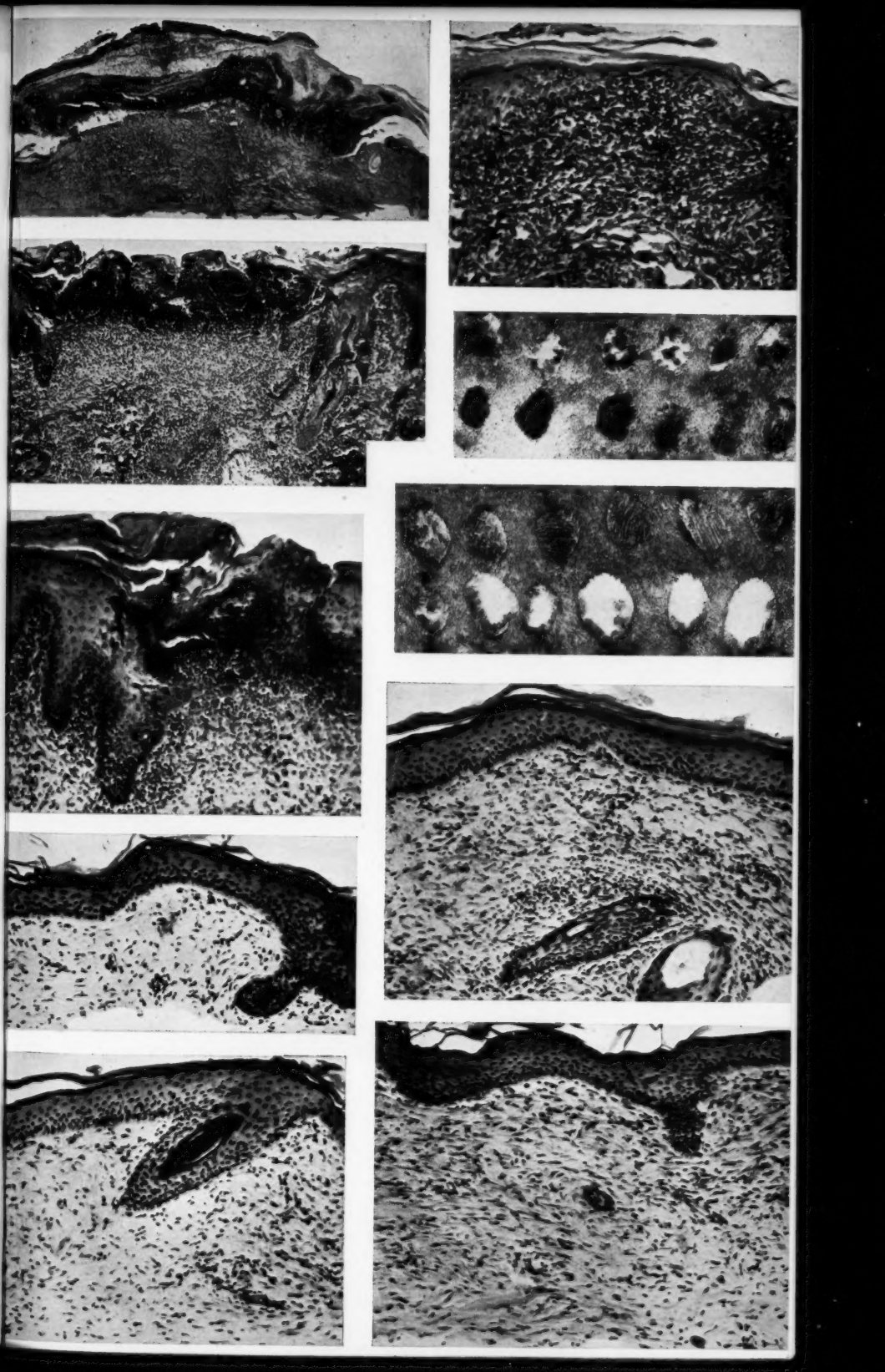
In fig. 14 (recipient: BH 3B) the top row of grafts are autografts, showing blotchy depigmentation: compare with their appearance as homografts in the bottom row of fig. 15 (recipient: BH 3A). The bottom row of grafts in fig. 14 are 6 homografts transplanted from BH 3A: compare with their appearance as autografts in the top row of fig. 15.

There has been complete survival of the homografts on both animals: the differences between the autografts on the one and the homografts on the other are presumably due in part to differences between the genotypes of the hosts. Natural size.

FIGS. 16 and 17.—Recipient: BH 1A, showing sections through ear skin grafts of 41 days' standing. Fig. 16 shows an autograft and fig. 17 a homograft transplanted from BH 1B. The latter shows a very minor perivascular round cell reaction (see text). ×100.

FIGS. 18 and 19.—Recipient: BH 1B, showing sections through ear skin grafts of 41 days' standing. Fig. 18 shows an autograft and fig. 19 a homograft transplanted from BH 1A. The two are virtually indistinguishable (see text). ×100.

All sections were stained with Ehrlich's hæmatoxylin and eosin.



each other's skin, and examples of chronic low-grade immunity reactions are reported.

It is suggested that the anomalous tolerance of dizygotic cattle twins to grafts of each other's skin has the same origin as their conformity of immunological blood types.

The work described in this paper was done at Cold Norton Farm (Stone, Staffordshire), one of the experimental farms of the Agricultural Research Council's Animal Breeding and Genetics Research Organisation. Miss Jean Morpeth gave valuable technical assistance throughout. In addition, we wish to thank Mr W. S. Biggar and Mr J. S. Tavernor for their friendly co-operation and practical help; and the work in all its stages has benefited greatly from thorough discussion with Dr H. P. Donald. The autopsies that verified the freemartin condition were done by Mr I. G. Shaw, M.R.C.V.S., to whom we are much obliged.

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STRUCTURE OF THE INCOMPATIBILITY GENE

III. TYPES OF SPONTANEOUS AND INDUCED MUTATION

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1. INTRODUCTION

PREVIOUS articles in this series have described the quantitative aspects of spontaneous and X-ray induced mutation of the incompatibility gene in *Prunus avium* and *Oenothera organensis* (Lewis, 1948, 1949). The present article deals mainly with the qualitative aspects of mutation in *Oenothera*. It describes the different kinds of mutation which have occurred, spontaneously and under treatment with X-rays, from four different **S** alleles.

Specific loci used for mutation studies in *Drosophila*, maize or micro-organisms usually have but one wild-type allele. The mutant alleles are recessive and are usually less efficient variants of the wild-type allele. The **S** locus in self-incompatible plants, on the other hand, has a large multiple allelic series, each allele having its own specific and positive effect. No one member rather than any other of this series can be looked upon as the " wild-type " allele ; rather should the whole series be thus considered. It should be possible, therefore to compare :—

(i) The mutational potentialities of different " wild-type " alleles
(ii) The mutations obtained with the " wild-type " alleles.
(iii) The kinds of mutations produced by X-rays with those arising spontaneously.

Genetical knowledge is still very sparse in regard to many aspects of mutation, and it is a matter of some urgency to fill out this knowledge in view of the present increase in man-made ionizing radiation. It is not only necessary to be able to compare induced mutations with the

spontaneous mutations which are found under present laboratory techniques but, what is more important, to compare both these types of mutations with the gene changes which have been used in the evolution of a balanced genotype ; these we might term *constructive mutations*. They may be the same as the spontaneous mutations we observe in the laboratory or they may be quite different. They may have such small phenotypic effects that our relatively crude methods of detection are useless. Or they may have effects, small or large, expressed only if the particular genetic system concerned is still actively evolving. If this speculation is true the difficulty again lies in our methods, for the genes which our mutation techniques can attack are genes which have big effects, *i.e.* genes nearing the end of their progressive evolution.

The data to be presented are on the changes of a gene which is probably highly evolved. Thus, if our speculation is true, they cannot be expected to reveal the mutational steps by which this gene has differentiated into its present polymorphic state. However, the work may serve to emphasise the necessity for caution when considering the kinds of changes which have been responsible for genic differentiation.

2. METHODS

Most of the methods have been described previously (*loc. cit.*) ; two refinements have been adopted during the present work, genotype testing and seed germination.

(i) *Genotype testing*

The S genotype of a plant was tested previously by self-pollination and reciprocal cross pollination with the six different test plants which are possible with four alleles, *e.g.* S2.3, S2.4, S2.6, S3.4, S3.6, and S4.6. This procedure requires thirteen pollinations for each plant to be tested.

It has now been reduced to four pollinations by using a tetraploid which carries the four different alleles S2.3.4.6. The plants to be tested are selfed, crossed reciprocally with their parent and crossed as pollen parents on to the tetraploid (see table 1).

The six different combinations of compatible and incompatible pollinations are shown in table 1. They are interpreted as follows :—

Results I and II prove that the seedling contains a mutant allele which causes self-compatibility.

The difference between results I and II when the seedling is crossed by parental pollen indicates the relationship of the mutant self-compatibility allele to the other allele in the seedling. For example it has been found that a self-compatible mutant allele retains its action in the style. Such an allele is designated with a prime suffix, thus S_a' is a self-compatibility mutant of S_a. A parent plant, S_{ab}

may have a mutation $S_a \rightarrow S_a'$, the S_a' allele would then pass to the seedling via the pollen. If the other allele of the seedling were S_a then the seedling would be S_{aa}' and this would be compatible with

TABLE 1

Method of genotype testing showing the four pollinations and the five different results

	I	II	III	IV	V	VI
Parental genotype * × Unknown seedling . S_{ab} × S_{as} or S_{bs} . .	+	+	−	−	+	+
Unknown seedling × Parental genotype . S_{as} or S_{bs} × S_{ab} . . .	+	−	−	+	+	+
Unknown seedling selfed . . . S_{as} or S_{bs} selfed	+	+	−	−	−	−
Tetraploid × Unknown seedling . . S2.3.4.6. × S_{as} or S_{bs} . .	+	+	−	−	+	−

* The parental genotype may have any pair (S_{ab}) of the four alleles S2.3.4.6. S_s is the unknown allele.

parental pollen S_b. So also will an S_{bb}' seedling be compatible with parental S_a pollen (result I). If on the other hand, the other allele is S_b then the seedling would be $S_a'_b$ and this would be incompatible with parental pollen (result II).

To identify which of the two alleles has mutated the seedlings giving result I or II have to be tested further. Seedlings S_{aa}' or S_{bb}' from result I are crossed by appropriate homozygotes if these are available or by testing whether all or a half of the pollen of test plants S_{ac} and S_{bc} is compatible on the seedling. Similarly if the seedling is S_{ab}' or $S_a'_b$, result II, crossing with S_{ac} and S_{bc} pollen will discriminate.

Results III and IV indicate that the unknown allele is similar to or identical with one of the parental alleles. The seedling is heterozygous in result III and homozygous in result IV. Again two further crosses will discriminate between the homozygotes.

Result V indicates that the unknown allele produces a full incompatibility reaction and is not one of the four alleles used in the experiments. It is a new fully operative S allele.

Result VI proves that the unknown allele is not one of the parental alleles but is one of the other two remaining alleles in the stocks. This may be a true mutation or a contamination by stray pollen from other genotypes. In all mutation work special methods must be used to guard against contamination from unwanted genes. Every precaution is taken in this work to isolate the clones in insect-proof greenhouses. But as a further precaution, only four alleles are kept in the stocks and the nearest plants of this species are 40 miles away. If the unknown seedling gives result VI in the test crosses it is

interpreted as a contamination. Admittedly *bona fide* mutations to the four stock alleles would be overlooked but this is trivial in view of the large number of **S** alleles in the wild population to which mutations might occur.

(ii) *Seed germination*

Many of the seeds obtained after self-pollination, particularly when there were only one or two seeds in the capsule, germinated with difficulty. The radicle emerged but the cotyledons failed to free themselves from the testa. The routine followed was to dissect off the testa and endosperm before germination and place the naked embryos on sterile pads soaked in Knopp solution in petri dishes. The dishes were placed in a greenhouse and additional light from fluorescent lamps was given for 5 hours each day. After 3-4 weeks the seedlings were ready to prick off into sand, and later into soil.

3. SPONTANEOUS MUTATION

The technique of using incompatibility as a means of detecting mutations of the **S** gene allows analyses of the mutations at three different levels of viability. At the lowest level there is the number of compatible pollen tubes in self-pollinated styles. This gives a maximum measure of the proportion of pollen grains with a changed incompatibility reaction, including those with an inviable sperm nucleus. The next level is given by the number of seeds produced. This gives an estimate of the number of pollen grains with a mutated **S** gene and having at the same time a sperm nucleus which is able at least to stimulate the initiation of a zygote. Finally, the number of seedlings produced gives an estimate of the **S** mutations which are fully viable. Only when the seedlings have been fully tested can we determine which type of mutation has occurred.

It has been shown by the pollen-tube method that there are overall differences between the **S** mutation rates of $S_4.6$ and $S_3.6$ genotypes (Lewis, 1948). The data in table 2 give the proportion of capsules which contain viable seeds after self-pollination in four genotypes. The fiducial limits for $p = 0{\cdot}025$ calculated by Stevens' (1942) method show that there are significant differences in the number of viable mutations produced by the genotypes. If we can detect which allele of the pair has mutated in each case it is possible to decide whether these differences in overall rates are due to differences in the rates of individual alleles.

In the tests made on the seedlings, if the new allele arising by mutation has some of its original specific properties, then we can attribute the mutation to a particular allele. Fortunately, from this point of view, all the alleles which have come through the mutation sieve can be related to their parental allele either because they still retain their function in the style unimpaired or because they are

apparently identical in all respects with their parental allele. The evidence for assuming the latter to be mutational products will be discussed later after all the data have been presented. Meanwhile they will be referred to as *revertible* mutations.

TABLE 2

Rates of fully viable spontaneous **S** *mutations obtained from four genotyes*

Diploid genotype	Flowers pollinated	Capsules with viable seeds	Mutations per 10^6 gene divisions *	
				Fiducial limits
S2.6	3,921	1	*0·02*	0·006-0·08
S3.4	931	5	*0·53*	0·17-1·25
S3.6	11,233	9	*0·08*	0·03 -0·15
S4.6	4,717	31	*0·65*	0·51 -0·82

* The number of gene divisions is calculated as $2Nk$, where N is the number of flowers, k is the number of pollen grains per stigma and 2 is a factor which adjusts the figures for the number of gene divisions occurring in the formation of the pollen. In using this factor it is assumed that the number of **S** genes increases in a geometric series as the power of 2. The last term in this series will be equal to the total of all the preceding terms. It is true that at some early stages in development some of the **S** genes will be diverted to other tissues and this will have the effect of making the estimate of the number of gene divisions in the descent of the pollen err in the low direction.

Revertibility applies to mutations from a stable allele to an unstable allele which soon reverts at a high rate to the normal. It is quite different from reversibility which applies to mutations from an allele **A** to an allele **a** which is as stable as the original allele but which can mutate back to **A** at a low frequency.

The mutations of all types obtained from four different alleles are given in table 3. It is apparent that, the four alleles show three distinct

TABLE 3

Spontaneous mutations from four alleles

	Flowers	Number of gene divisions	Number of mutations	Rate per 10^6 gene divisions	Fiducial limits
S2	3,921	$19·6 \times 10^6$	0	...	0 -0·05
S3	12,164	$60·8 \times 10^6$	0	...	0· -0·05
S4	5,648	$28·2 \times 10^6$	27	0·9	0·73-1·21
S6	19,871	$99·3 \times 10^6$	19	0·19	0·13-0·25

levels of mutability. The rate obtained with S6 is 0·19 and with S4 it is 0·9 per 10^6 gene divisions.

Assuming that S2 and S3 have a zero mutation rate, calculations

of the expected numbers of mutations to be obtained from the genotypes and numbers of tested alleles given in table 2 are as follows :—

	$S_{2.6}$	$S_{3.4}$	$S_{3.6}$	$S_{4.6}$
Observed . .	1·0	5·0	9·0	31·0
Expected . .	3·7	4·2	10·5	25·5

The good fit shows that the differences between the genotypes are due to the different mutabilities of the alleles and not to differences in other genes which might modify the mutation rate of the **S** gene.

Plants derived from the revertible mutations were themselves used in self-pollination to test the mutation rates of the alleles which were derived from one, and in some cases, three generations of selfing.

The data for the derived alleles are given below :—

Allele	Flowers	Gene divisions	Mutations	Mutation rate per 10^8 gene divisions	
					Fiducial limits
S_4	339	$2{\cdot}15 \times 10^8$	2	0·9	0·11-3·35
S6	803	$4{\cdot}01 \times 10^8$	2	0·4	0·06-1·80

Clearly these alleles are as stable as their predecessors and there is no sign of a progressive change to a new allele.

In table 4 the revertible mutants have been classified according to the number of seeds in each capsule. This indicates the number

TABLE 4

The number of capsules obtained after self pollination, classified according to the number of seeds they contain : for explanation of the expected ratios see the text

Seeds per capsule	Nuclear divisions	Number of capsules	Expected ratios with constant mutation rate	
1	0	15	23·2	13·12
2	1	3	11·6	6·56
3- 4	2	5	5·8	3·28
5- 8	3	12	2·8	12·24
9-16	4	6	1·4	6·12
17-32	5	3	0·7	3·06
33-64	6	2	0·3	1·53

of nuclear divisions occurring during the time from mutation to the formation of the pollen grains. In other words it is an estimate of the

number of gene divisions occurring between the mutation and the test made to detect it.

It is known that ovaries with less than five fertilised ovules are rarely stimulated to develop, except with the aid of growth-substances. The expected ratios given in columns 4 and 5 of table 4 are based on a constant mutation rate. In column 4 the classes are taken all together irrespective of whether there is any difference between the classes in the viability of the seeds. The fit is not good. In column 5 the classes have been divided into two groups with the 3-4 and 5-8 classes as the dividing line. Here the fit to expectation is much better and is particularly good in the last four groups. These are the groups which should have equal viabilities. The data as a whole therefore fit the expected series and support the revertible mutation hypothesis.

Apart from this the main point of interest is that the revertible mutation can cause a loss of genic activity which will persist for as much as six cell divisions and then at sometime during the growth of the embryo and plant recover its full activity.

There are two exceptions to the general rule that spontaneous mutations are of the revertible type.

(i) A family of plants was obtained from an S2.6 clone by self pollination without treatment. This family contained three seedlings each having a stable S6′ allele. This type of mutation has been frequently found after X-rays.

(ii) A family of five plants was obtained from an S4.6 clone. One plant carried a stable S6′ allele while the others had the reverted S6. This exception is of special interest for the explanation of revertible mutations.

Discussion of the significance of this exception is deferred until after the X-ray results have been described.

4. X-RAY INDUCED MUTATION

Doses of X-radiation ranging from 500-700 *r* units have been given to five different S genotypes. The pollen which subsequently developed from the treated buds was placed on stigmas of the same clone. The frequencies and kinds of mutations obtained are given in table 5. There were two kinds of mutations; the permanent loss mutations and the revertible mutations.

The permanent loss mutation S6→S6′ has occurred six times. All six S6′ alleles are identical in behaviour and differ from the original S6 allele only in their lack of action in the pollen grain. No incompatibility reaction is developed in the pollen grain carrying S6′ but all other characters, such as style reaction, viability, etc., are unaffected. A full description of the tests made on the original S6′ mutation have been described (Lewis, 1949).

A new example of this type of mutation was found in 1950 from an S3.4 plant which had received 700 *r* of X-rays. Two families were obtained, one containing 4 and the other 10 plants. Both families

TABLE 5

Types and numbers of different mutations produced from five genotypes by X-radiation (dose 500-700 r)

Diploid genotypes	Flowers pollinated	Permanent loss mutations		Revertible mutations	
		Type of mutation	Number	Type of mutation	Number
S3.6	870	S6 → S6′	6	S6 → S6	7
		S3 → —	0	S3 → —	0
S2.6	64	S6 → S6′	0	S6 → S6	1
		S3 → —	0	S3 → —	0
S4.6	237	S6 → S6′	0	S6 → S6	1
		S4 → S4′	0	S4 → S4	2
S2.3	117	S2 → —	0	S2 → —	0
		S3 → —	0	S3 → —	0
S3.4	67	S3 → —	0	S3 → —	0
		S4 → S4′	2	S4 → —	0
Total			8		11

had approximately half the plants compatible and half incompatible when used as females with their parents. All the plants were self fertile, and all were compatible as males on to their parent. The plants which were compatible as females were found to be S4.4′ by crossing to a known S4.4 test plant and the plants which were incompatible were S3.4′. Thus the new mutation of S4→S4′ involves only a loss of the pollen activity exactly as in the S6′ mutations and they are classified as permanent loss mutations.

The revertible mutations induced by X-rays are also from S6 and S4. These are similar to the spontaneous revertible mutations described in the previous section.

No mutations of S2 or S3 either permanent or revertible have been obtained with or without X-ray treatment.

If a comparison is made in table 5 of frequency of permanent loss mutations, S6′ and S4′, obtained from different diploid genotypes it will be seen that the allele which accompanies the mutating allele appears to influence the direction of mutation. For example S6 and S4 both give permanent and revertible mutations, but all the stable S6′ and S4′ mutation have come from S3.6 and S3.4 plants, that is, when the mutating allele is accompanied by S3. The genotype S4.6, despite having both potentially mutating alleles has produced no permanent mutations. A calculation of the number of expected S6′ and S4′ mutations from the number of pollinations of S4.6 is as follows :—

$$\frac{6\times237}{870}+\frac{2\times237}{67}=8{\cdot}6$$

which is also the χ^2 for such a deviation with a probability of less than 0·01.

On the other hand the frequency of revertible S6 and S_4 mutations obtained from the S6.4 genotype is not lower than the expected value obtained from the S_3.4 and S_3.6 genotypes. This is in agreement with the results from spontaneous mutation.

In order that a comparison can be made between the induced and spontaneous mutations, the data are arranged for the 4 alleles in table 6.

TABLE 6

Mutation rates per 10^6 S *genes with* 500-700 *r units of X-rays compared with the spontaneous rate* (R = *revertible*)

Allele	Number of alleles treated *	Mutation type and number	Rate per 10^6 genes	
			Induced	Spontaneous
S6	$1{\cdot}46\times10^6$	S6 (R) 9	5·4	0·19
		S6′ 6	4·1	0·01
S_4	$0{\cdot}38\times10^6$	S_4 (R) 2	2·1	0·9
		S_4' 2	5·2	0·0
S_2	$0{\cdot}08\times10^6$	S_2 (R) 0	0	0
		S_2' 0	0	0
S_3	$1{\cdot}17\times10^6$	S_3 (R) 0	0	0
		S_3' 0	0	0

* The number of treated alleles is difficult to estimate owing to the presence of cells in different stages of development. The estimate made is half the number of alleles tested, this is an overestimate so the induced rates err on the low side.

X-radiation has raised significantly the frequency of both S6′, S_4' and of the revertible S6 mutations.

The frequency of S_4 revertible mutations is higher after X-rays but not significantly so.

One further point to consider with the induced mutations is the relationship between the stage of development of the anther tissue at the time of irradiation and the number of seeds produced in each capsule.

It is necessary to recall that the flowering shoots of *Oenothera* have a graded developmental series of buds, the youngest being at the tip and the oldest at the base of the shoot. The flowers open at daily intervals so that flowers which open soon after irradiation were irradiated at a late stage of development, those opening later were irradiated at an earlier stage. If a mutation has been induced in a late stage of pollen development there will be only one mutated pollen grain and hence one seed in the capsule. The earlier in development the mutation is induced the higher will be the number of mutated pollen grains, hence the larger the number of seeds in the capsule. In fig. 1 the data have been plotted according to the number of seeds per capsule and the number of days from X-ray treatment to flowering.

These data have been collected over a number of years and in consequence the conditions affecting the irradiated plants would be far from constant. The general rate of flower development is dependent on temperature and other conditions, so that it is to be expected that there will be considerable deviation from the expected relationship between time and the stage of development as estimated from the number of seeds produced. This deviation, however, is not enough to obscure the main issues.

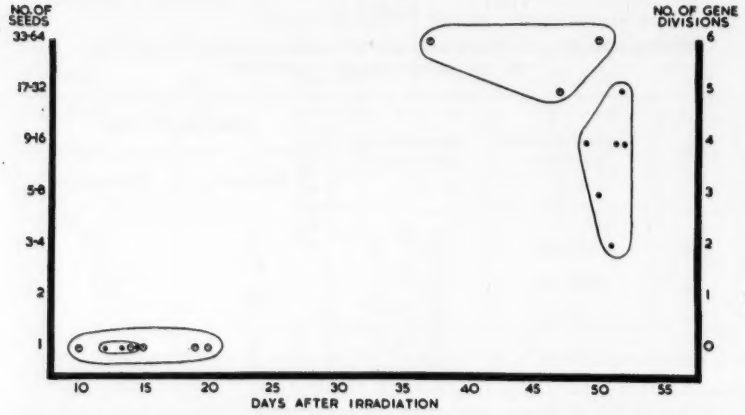

FIG. 1.—The relationship between the number of seeds in the capsules and the time in days from irradiation to pollen maturity. The number of seeds is determined mainly by the number of gene divisions intervening between irradiation and pollen maturity. ● represents *revertible* mutations, ⊙ represents the *permanent* loss mutations.

The single-seeded capsules occur when the interval between irradiation to flowering is 10-20 days. This is true for both permanent loss and revertible mutations.

The capsules with 3-64 seeds occur over a period of 37-52 days after irradiation. Here it is possible to see that the two types of mutations, permanent loss and revertible, occupy different areas of the graph. The significance of this difference will be referred to in the discussion on the nature of the revertible mutations.

The main point to be made here is : that the capsules containing a single seed and those containing many are sharply separated in days after irradiation. This applies both to the permanent loss, S6′ and S_4' mutations and also to the supposed revertible mutations. Thus not only have X-rays produced a significant increase in the revertible mutations over the controls, but these occur in numbers which are in keeping although not identical with the numbers of true loss mutations occurring at different times after irradiation.

5. PSEUDO-FERTILITY OR REVERTIBLE MUTATION ?

The main problem arising out of the present work is the explanation for the functioning in self-pollination of what appear in the next generation to be unchanged alleles. One fact of considerable importance in this connection is the extreme rarity of the phenomenon. For every $2{\cdot}2 \times 10^6$ untreated pollen grains applied to stigmas of *O. organensis* only one or one small group of pollen grains functions. This frequency is commensurate with mutation rates and is lower than would be expected if the cause lay in some environmental effect on incompatibility.

Furthermore, out of the four alleles used only two, S6 and S_4 have shown this temporary inactivation, and treatment with X-rays has also produced mutations only in these alleles. These induced mutations have been of a genuine stable loss type and also of the spontaneous type under discussion. This latter type do not occur at random in time but the numbers are related to the length of time between irradiation and pollen maturity as would be expected if they were true genic changes.

Can these rare S6 and S_4 pollen grains which are compatible on their own style be thought of as due to *pseudo-fertility ?*

Pseudo-fertility, as known in other species, has been attributed, to several causes—end-of-season condition, bud pollination and modifier genes which weaken the incompatibility reaction. The most detailed study on *pseudo-fertility* was made by East (1934) on *Nicotiana Sanderæ*.

He showed that different genotypes had different degrees of pseudo-fertility and that it was increased by pollination in the bud stage. The number of seeds in a capsule after selfing was often as much as one-half and never less than one-tenth the normal compatible complement. The families resulting from pseudo-fertility contained both S homozygotes, showing that both S alleles had functioned on the male side.

In *O. organensis* Emerson (1940) has shown that there is no end-of-season or bud fertility. It is true that allelomorphs S_9 and S_{39} were found to produce incompatible pollen tubes that were longer than normal but no seed was produced. Emerson found also that keeping the plants in the dark weakened the incompatibility reaction slightly but again no seeds were produced. In the course of my own work I have found a slight end-of-season fertility as measured by pollen-tube growth but again there was no effect on seed set.

On the other hand the following summary points to some temporary, and for the time complete, loss of activity of the S allele.

(i) The low frequency of its occurrence.
(ii) The low number of seeds when setting does occur.
(iii) The increase in frequency by X-rays.
(iv) The absence of simultaneous effects on both alleles.

If a loss of S activity is the cause then it must persist in some cases for at least six divisions to account for the capsules with more than 33 seeds. The activity must be restored at some time between fertilisation and the formation of the next generation pollen-mother-cells to account for the return to the old allele when tested.

What are the likely causes for the loss of the S gene activity?

1. Mutation of a complementary gene or a modifier which when accompanied by a rare combination of other segregating modifiers weakens the action of the S gene.

This explanation must be rejected because there would be a great increase in self-fertility in the next generation, and this has not been found.

2. Pollen grains having a vegetative nucleus with a mutated S allele and a generative nucleus with a normal allele.

This would explain the occurrence of single seeds but would not explain the presence of groups of seeds in some capsules.

3. A possible cytoplasmic component of the system could temporarily be lost from a cell.

This component would have to be present in meiotic and pre-meiotic cells to explain the loss of S activity from a group of pollen grains.

For the present argument it is important to distinguish between two possible cytoplasmic components :—

(i) A non-specific one, that is, one which is a common substrate for all the S alleles; the alleles producing their specific effects in combination with the component after meiosis.

(ii) Components which are specific to each allele. In this case a meiotic or pre-meiotic cell would contain two different kinds of components, one for each S allele in the cell.

Whatever type of cytoplasmic component is present it would be distributed to the four cells of the tetrads. If the component is specific to each allele then the irregular segregation which is characteristic of cytoplasmic particles would result in a considerable proportion of tetrad cells receiving only the wrong components. This would lead to a breakdown of the system. Hence, we are left with the possibility of a non-specific component.

A non-specific component would have to be present in numbers sufficient to ensure that all cells of the tetrad always received some. It is unlikely, therefore, that all the components in a cell would be lost by 700 *r* units of X-rays.

The crucial test, however, for such a hypothesis is that the loss of a non-specific component in a pre-meiotic cell would mean the simultaneous loss of activity of both the S alleles in the cell.

This would result in mixed families of both homozygotes. These have not been found but unfortunately the numbers tested are not sufficient to decide whether this is due to errors of sampling.

4. The fourth possibility is of a revertible mutation of the S gene.

This explanation is the one that fits the facts better than any other. The assumptions needed on this basis are (i) that an S allele mutates to an allele which is inactive at least as far as the pollen action is concerned, and (ii) that after some gene reproductions it reverts to its normal activity. The maximum number of gene reproductions to reversion is unknown, but it is known that reversion can occur after as many as six reproductions in the unstable state.

Further evidence for such a reversion is given in fig. 1. In this diagram the relationship between the time after irradiation to pollination and the number of seeds produced is plotted. It will be seen there that for the many-seeded capsules the area covered by the revertible mutations is different from that covered by the permanent loss mutation. The latter, though they arose at a longer interval after irradiation, appear to result from fewer intervening divisions. This could be explained by a reversion of some of the revertible mutants *before* pollination, so that the number of seeds would correspond to less gene divisions than had actually occurred.

One family of seedlings obtained without X-ray treatment is of great significance here. From selfing an S4.6 clone a family composed of plants which were S6.6 and one plant which was self-fertile S6.6′. Thus there were revertible and permanent loss mutations in the same family. It seems that the S gene can mutate to an unstable or segregating condition which can either revert to the old condition or stabilise in the new condition.*

The obvious parallel between this conclusion and the results obtained by Auerbach (1950) from mutations obtained by chemical treatment in *Drosophila* indicates that perhaps the distinction between spontaneous, X-ray and chemical mutations is indeed small.

Auerbach favours the view that chemical treatment may induce a labile premutation which subsequently, after one or more cell divisions, may give rise to the actual mutation or may revert to the old allelemorph.

Darlington (1950) considers the chromosomes to be composed of a number of polypeptide chains, and that delayed mutation is due to the sorting out of mutated from unmutated chains.

The delayed mutations produced by X-rays in *Drosophila* are usually of the half and half mosaic type and these are due to a delay of only one division.

They are readily explained by assuming that two nearby chromosome breaks have occurred and that after the chromosome has split, there has been restitution in one chromatid and a deletion in the other (Muller, 1940).

In bacteria, Demerec and Latarjet (1946) have found that X-rays produce mutations which may not be manifested for as long as 13

* The rare possibility cannot be excluded of a contamination from an extraneous source of S6′ pollen coincidental on the same stigma with the genuine revertible S6 pollen.

divisions. Their explanation lies not in delayed mutation but delayed phonotypic expression.

The only reported cases of delayed mutation after X-rays are, therefore, the one division delays in *Drosophila*. This would appear to weigh heavily against the delayed mutation explanation in *Oenothera*. However, the chromosomal and mutational effects obtained with X-rays are so different quantitatively and to some extent qualitatively in different tissues that this objection is not serious.

6. CONSTRUCTIVE AND LOSS MUTATIONS

Mutation studies, in the past, with their focus on *Drosophila* have been concerned with lethal mutation in a large number of unidentified loci or with changes from a dominant to a recessive allele of a few selected loci. Both these kinds of mutation represent loss changes. Recently attention has been directed to mutations from the recessive back to the dominant " wild-type " allelemorph in *Drosophila*.

These experiments have given entirely negative results (Lefevre, 1950).

With the shift of focus to micro-organisms in the last decade mutations from both wild type to recessive and the reverse have been studied on a much larger scale than has been possible with *Drosophila*.

In *Neurospora crassa* reverse mutations of four genes have been found, but with other genes no reverse mutations could be found or induced (Ryan and Lederberg, 1946 ; Giles and Lederberg, 1948 ; Kölmark and Westergaard, 1949). These reverse mutations are the regaining of the ability to produce a " wild-type " character by an allele which previously had lost this ability. This always leaves the loophole of position effect as an explanation. And in this connection it is significant that the back mutations not only occur spontaneously but are induced by X-radiation and ultra-violet light.

All the mutations, therefore, are either losses of wild type to recessive mutant or a regain of such losses in back mutations. There is nothing new and constructive.

The **S** gene, with its many alleles each having a specific and different effect, would appear to be pregnant with possibilities for constructive mutations. For if the existing alleles in their hundreds have arisen in nature by a mutational process of the kind observed under present experimental conditions then new alleles should arise in experiment.

That none have arisen from 220×10^6 gene divisions which have received no treatment and from $3{\cdot}1 \times 10^6$ which have received 600 *r* units of X-rays throws doubt on the fundamental principles underlying the experiments.

Perhaps constructive mutations detectable with present techniques do not occur.

7. SUMMARY

1. Four alleles of the incompatibility gene in *Oenothera organensis* have been tested for mutation. Two of these alleles have not mutated, either spontaneously or after X-irradiation, in a total of 80×10^6 gene divisions tested. The two other alleles have given two types of mutation : *permanent* and *revertible* mutations.

2. All the permanent mutations involve a loss of the pollen action of the gene only. The stylar action is unimpaired.

3. The revertible mutations enable the pollen grains immediately after mutation to pass the incompatibility test. But these mutations have no effect when tested in the next generation some 15 or 20 divisions later.

4. These temporary changes are probably caused by S mutations to an inactive but unstable form which reverts, either to the normal active allele, or to a stable inactive allele as in the permanent mutation. A cytoplasmic modification cannot, however, be entirely dismissed.

5. Both permanent and revertible mutations occur spontaneously and are induced by X-rays. X-rays induce both types with about equal frequency. Spontaneously the permanent mutations are, however, rarer than the revertible ones.

6. No constructive mutations, *i.e.* to new fully functioning incompatibility alleles have occurred in 223×10^6 gene divisions tested.

7. Tests for small and cumulative changes were made by self pollination for three generations on plants which had arisen from the revertible mutations : no changes in the alleles could be detected.

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THE INFLUENCE OF THE Y-CHROMOSOME ON QUANTITATIVE CHARACTERS OF *D. MELANOGASTER*

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1. INTRODUCTION

LITTLE is known about the genetical action exerted by the heterochromatin in general, and by the Y-chromosome in particular. The information available is so scanty, that many scientists consider heterochromatin as inert, *i.e.* devoid of genetical activity.* Nonetheless some investigations indicate that a peculiar kind of control on certain characters is exerted also by the Y-chromosome on abdominal chæta of *Drosophila melanogaster* (Mather, 1944), less clearly on variegation (Demerec, 1940, 1941 ; Schultz, 1941, 1947), and finally on the wing hairs, and other small characters (Barigozzi, 1948, 1949). In other species, a genetical activity was proved for the heterochromatin of *Sphærocarpus* (Knapp, 1935), of *Sorghum* (Darlington and Thomas, 1941) and of *Anthoxanthum* (Oestergren, 1947). Also in some *Rynchota*, Darlington (1939) put forward a possible action of small heterochromatic elements. Following a completely different line, Caspersson and Schultz (1938) and Caspersson (1940) gave some evidence for the presence of a centre for protein and polynucleotide synthesis in the heterochromatin. According to this view, we have a second reason to believe that heterochromatin is not inert.

No more than an introduction to the knowledge of the heterochromatin role has thus been worked out as yet. Even though heterochromatin is likely to produce only small effects, we do not know the kind of characters affected by it.

The present paper,† which includes some data previously published (Barigozzi, 1948, 1949 ; Di Pasquale, 1951) should be a contribution to the knowledge of such characters which, differing between male and female, can be supposed to be controlled by the Y-chromosome, known as completely heterochromatic.

2. MATERIAL, METHODS AND TECHNIQUE

The characters chosen for the present investigation have been the following : (1) frequency of unicellular hairs of a given portion of the wing, (2) size of the corneæ of the compound eyes, (3) irregularities

* The few major genes localised in the Y, as *bobbed*, are not considered here.

† For the statistical elaboration, I am indebted to the courtesy of Professor K. Mather, who gave me valuable advice.

of distribution in the corneal complex of the compound eyes. All these characters are alike in being formed from a fixed number of cells. In fact, each wing hair corresponds to one single cell (Dobzhansky, 1929). The corneæ are likely built from two cells, and the irregularities of distribution are due to the presence of single corneæ of unusual size (Di Pasquale, 1951). This means that a common factor plays a role in all these characters, *i.e.* the cell size. The action exerted by the Y-chromosome was detected by means of stocks, constructed in order to have each a peculiar Y and a set of the remainder chromosomes identical in all stocks. The Y's were obtained from the wild stocks: Oregon R, Oerlikon, Crkwenika, and Luino. The other chromosomes came for all the stocks from Oregon R. Various Y-chromosomes were placed on a background of Oregon chromosomes ("oregonised") by the following two procedures.

1. Repeated backcrosses of heterozygous males to Oregon females. According to Mather (1944), the oregonisation can be considered practically complete after 7 generations of backcrosses, if single males are used.

2. Substitution of full chromosomes by means of crosses between the Oregon stock, which shall provide all the chromosomes except the Y, and a stock with the X and autosomes marked with dominants and provided with structural changes preventing crossing-over. In the present investigation the procedure was developed in the following manner :—

(*a*) Construction of stocks with the desired Y and marked chromosomes.

$$\frac{Y^{Luino}}{+}\ \frac{Pm}{Cy\ L}\ \frac{Sb\ M\acute{e}}{H};\ \frac{C1B}{+}\ \frac{Pm}{Cy\ L}\ \frac{Sb\ M\acute{e}}{H}\times$$

$$\frac{Y^{Crkwenika}}{+}\ \frac{Pm}{Cy\ L}\ \frac{Sb\ M\acute{e}}{H};\quad ,,\quad \text{or}$$

(*b*) $$\frac{Y^{Luino}}{+}\ \frac{Pm}{Cy\ L}\ \frac{Sb\ M\acute{e}}{H}\times\frac{x^{Or}}{x^{Or}}\ \frac{II^{Or}}{II^{Or}}\ \frac{III^{Or}}{III^{Or}}$$

$$\frac{Y^{Luino}}{x^{Or}}\ \frac{Pm}{II^{Or}}\ \frac{Sb\ M\acute{e}}{III^{Or}}\times\frac{C1B}{x^{Or}}\ \frac{Cy\ L}{II^{Or}}\ \frac{H}{III^{Or}}$$

(*c*) $\frac{Y^{Luino}}{X^{Or}}\ \frac{II^{Or}}{II^{Or}}\ \frac{III^{Or}}{III^{Or}}$, and the same for the other combinations.

This technique is based on the assumption that the Oregon-R stock is practically isogenic. A disadvantage lies in disregarding the IVth pair. But a comparison between the results obtained with method (1) and (2) permits the conclusion that no appreciable difference exists between the methods.

In this manner the way is open to compare individuals with Y

(males) and individuals without (females). The remainder of the genome being identical, every discrepancy in sex difference must be ascribed to differences of the genes contained in the Y-chromosome, to the difference in gene dosage, when a single X or two X's are present, or to a maternal effect.

The counting of the hairs was made on wings detached from the individual and mounted in glycerine. The surface within which the counting was made, was determined by a reticulum introduced into the eye-piece of the microscope. In all observations, the magnification has been of *circa* 960. The hairs were counted considering the emerging points of those hairs comprised in two adjacent squares, adjusting the preparation to show the region located immediately below the first crossvein, on the superior surface.

The corneæ were observed detached from the eye after treatment with aqueous KOH solution, and mounted in glycerine.

The measurements of the corneæ (expressed in μ^2) were made with the help of the camera lucida, with a magnification of *circa* 950 diameters. For each individual, 10 corneæ were measured and then the mean was calculated. The irregularities—whose frequency was also counted—resulted from differences in size of corneæ, producing disorders of the corneæ distribution, in individuals of the genotype

$$\frac{Sb\ cr\ /n\ (3R)\ M\acute{e}}{III^d\ Chr}$$ (Di Pasquale, 1951).

The statistical treatment was made using the following methods :—

1. The comparison between the means of the hair frequencies was made with the σ_d or error of the difference, where the number of individuals was not less than 50.

In one case (Luino), for which only smaller samples were used, the comparison was made by the analysis of the variance.

2. When the individuals did not reach 50, the means were compared with the t test.

3. The frequency of the irregularities of corneæ location follows a Poissonian-like distribution. The comparison between sexes was made with the χ^2, grouping the classes in order to eliminate those with a frequency less than 5.

3. OBSERVATIONS

A.—Four wild stocks were investigated for the characters : hair frequency, corneal size, frequency of irregularity of the corneæ. Here are the data and the statistical treatment as indicated, for testing the significance of the difference between sexes.

Oregon

1. *Hairs.*—This stock has been investigated twice and the following figures have been obtained.

Number of hairs . .	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
First sample of 28 ♀ and 30 ♂																
♀	1	...	1	2	4	6	4	1	4	3	2	...	...	...	...	...
♂	...	...	...	...	...	...	1	2	1	7	4	7	4	1	3	1
Mean ♀ 15·86 Mean ♂ 20·40	difference 4·55 ; $t = \frac{4{\cdot}55}{0{\cdot}6045} = 7{\cdot}5269$; $\nu = 56$; P<0·01 significant															
Second sample of 100 for each sex																
♀	2	3	19	28	19	18	6	4	1	...	...	...	...	...	...	...
♂	...	...	1	8	6	18	22	18	16	5	4	2	...	...	...	...
Mean ♀ 14·63 Mean ♂ 17·33	difference between means : 2·70±0·239 significant *															

* The strong difference between the means is explained by the time which occurred between the countings. The same phenomena will be observed for the corneal irregularities see later).

2. *Corneæ.*—Thirty individuals for each sex (see table 1) :—

TABLE 1

Surfaces in μ^2 of the corneæ of Oregon

♂					♀				
538	664	708	769	815	643	753	800	892	939
587	668	710	776	816	654	762	814	902	955
646	677	729	795	834	692	767	822	910	963
647	679	729	795	845	693	772	826	913	983
657	681	760	801	846	728	778	872	928	983
663	689	761	811	864	741	785	891	939	1127
Total		30					30		
Means		732·0					840·9		

Difference 180·9 ; $t = \frac{108{\cdot}9}{25{\cdot}53} = 4{\cdot}265$; $\nu = 58$; P>0·01. The difference is significant.

3. *Irregularities of the corneæ* in individuals $\frac{Sb\ cr\ In\ (3R)\ M\acute{e}}{III^{d}\ Oregon}$ 50 for each sex :—

Number of irregularities	♀	♂	Total	χ^2
0	47	29	*76*	4·263
>0	3	21	*24*	13·500
Mean	0·06	0·66	...	17·763

Difference 0·60 significant ; $\chi^2 = 17{\cdot}763$; $\nu = 1$; $P > 0{\cdot}01$

One remarks that in all the investigated characters, the difference between the sexes is significant.

Oerlikon

1. *Hairs.*—One hundred for each sex.

First sample 1948

Number of hairs .	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	Means
♀ . . .	2	10	16	29	21	17	2	3	...	...	...	15·31
♂ . . .	...	...	1	2	4	15	24	27	17	7	3	18·61
												Difference 3·30±0·209

The differences between sexes is significant

Second sample 1949

Number of hairs .	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	Means
♀ . . .	...	3	7	18	27	29	11	4	...	1	...	16·27
♂ . . .	1	...	3	4	16	29	17	17	7	3	3	17·71
												Difference 1·44±0·227

The difference is significant.

If we compare the means of the samples, testing for the hair frequencies the standard error of the difference, we obtain :—

Difference between females : 0·96±0·204
Difference between males : 0·90±0·231

We can conclude that the difference is significant, since the error does not reach the third of the difference.

2. *Corneæ* not measured.

3. *Irregularities of the corneæ* in individuals $\frac{Sb\ cr\ In\ (3R)\ Mé}{III^d\ Oerlikon}$:—

(*a*) *First sample*, 1948, *of* 61 *individuals for each sex*

Number of irregularities	♀	♂	Total	χ^2
0	46	35	*81*	1·493
1-2	10	8	*18*	0·222
>2	5	18	*23*	7·347
Mean	0·67	1·57	...	9·062

Difference 0·90 significant ; $\chi^2 = 9·062$; $\nu = 2$; P = 0·02-0·01

(*b*) *Second sample*, 1949, *of* 61 *individuals for each sex*

Number of irregularities	♀	♂	Total	χ^2
0	28	27	*55*	0·018
1-2	9	11	*20*	0·200
3-4	12	7	*19*	1·315
>4	12	16	*28*	0·571
Mean	2·09	2·36	...	2·104

Difference 0·27 not surely significant ; $\chi^2 = 2·104$; $\nu = 3$; P = 0·70-0·50

One remarks that in two subsequent years the difference between sexes is decreased for both characters, more clearly for the corneal irregularities, although both characters showed formerly a significant difference between sexes.

Crkwenika

1. *Hairs.*—One hundred individuals for each sex.

Number of hairs . .	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*
♀	1	...	4	14	17	25	16	14	5	3	...	1
♂	...	...	...	4	8	22	18	17	10	15	5	1

Mean ♀ 15·15 Difference 1·42±0·258 significant
Mean ♂ 16·57

Corneal size and irregularities not investigated.

LUINO

1. *Hairs.*—One hundred individuals for each sex.

Number of hairs	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
♀	1	...	2	...	2	7	14	15	18	12	11	4	8	2	2	...	1	1
♂	...	...	1	1	3	5	6	12	17	15	16	11	8	3	...	...	...	2

Mean ♀ 21·25 Difference 0·58±0·377 not significant
Mean ♂ 21·83

2. *Corneæ.*—Thirty individuals for each sex (see table 2) :—

TABLE 2

Surface in μ^2 of the corneæ of Crkwenika

♂					♀				
502	607	678	733	795	542	601	652	701	762
518	611	678	742	876	547	606	657	710	782
580	626	693	742	887	551	629	657	715	793
588	630	699	743	887	578	643	686	731	803
603	635	727	752	943	582	648	696	731	808
603	635	733	753	949	601	648	701	749	896
Total		30					30		
Means		704·933					680·200		

Difference 24·733 ; $t = \frac{24·733}{26·675} = 0·927$; $\nu = 58$; $P < 0·4$. Difference not significant.

3. *Irregularities of the corneæ* in individuals $\frac{Sb\ cr\ In\ (3R)\ M\acute{e}}{III^d\ Luino}$ not investigated.

One remarks that the difference between sexes is not significant in both the tested characters.

4. GENERAL CONCLUSIONS ON THE WILD STOCKS

1. The most general fact observed is the higher cellular size in the female. This is demonstrated by the smaller frequency of unicellular hairs in a given surface of the wing and the greater size of the corneæ.

2. The difference between sexes is different in different stocks, being significant for all the considered characters. The difference is significant in Oregon, Oerlikon and Crkwenika, and not in Luino.

The possible explanation for the phenomena is that the Y- or the X-chromosomes could be responsible of a control on the characters under consideration.

To decide what an alternative may be true, the following experiment has been designed : to bring the Y of one stock with a low sex difference into one stock where the difference is high, or *vice versa*. Since the stocks showing the most striking discrepancy in sex difference are Oregon R and Luino, it seemed opportune to bring together the Y of Luino with the other chromosomes of Oregon. The expectation is :—

(*a*) If Y plays a prominent role in controlling the considered characters, the combination of Y of Luino with the Oregon set will produce the insignificant difference between sexes, which seems typical for Luino.

(*b*) If that is not true, and different parts of the genome take part in the control, the sex difference of Luino will not be observed.

The following data have been obtained after two independent oregonisations with the method of the repeated crosses (see page 416), in order to ascertain if the procedure can give homogeneous results. The stock obtained is labelled " Luino oregonised."

Hairs.—From these data the analysis of the variance was used to prove if the 4 samples, two of males and two of females, obtained in both oregonisations belong to the same population.

Number of individuals.—First sample : 30 for each sex. Second sample : 15 for each sex.

First sample			Second sample		
Number of hairs	♂	♀	Number of hairs	♂	♀
14	1	...	*15*	...	2
15	...	...	*16*	4	...
16	4	3	*17*	4	3
17	6	4	*18*	4	4
18	8	11	*19*	3	5
19	9	4	*20*	...	...
20	2	5	...	...	...
21	...	3	...	...	...

As a test of homogeneity of the two stocks obtained through independent oregonisations the frequency of hairs of which is given in the following table, an analysis of variance was carried out. The results of the analysis given below showed no significant heterogeneity.

Analysis of variance

Variance between four series	df 3	SS 11·94	var. 3·98	F 2·101	P>5 per cent.
Variance between sexes	1	4·90	4·90	2·587	>5 ,,
Residual (sex difference + interaction)	2	7·04	3·52	1·859	>5 ,,
Variance within samples	86	...	1·894	...	...

Corneæ.—First sample : 29 individuals for each sex (see Table 3). Second sample : 15 females, 13 males.

TABLE 3

Surfaces in μ^2 of the corneæ of "Luino oregonised"

♂					♀				
657	720	786	840	922	692	752	795	871	931
666	724	800	855	926	705	761	804	888	949
668	732	800	886	928	726	762	836	893	965
694	755	817	894	948	729	772	836	895	994
695	766	825	905	951	750	789	839	913	1033
710	778	839	913	...	752	793	841	930	...
Total		29					29		
Means		806·98					834·34		

Difference 27·45 ; $t = \frac{27{\cdot}45}{23{\cdot}42} = 1{\cdot}17$; $\nu = 56$; P = 0·3. Difference not significant.

The second sample gave the following means : females, μ^2 738·64 ; males, μ^2 744·77 ; difference, μ^2 5·07. Since the difference in the first sample is not significant, and in the second one it is even less, no further statistical calculation has been carried on.

Irregularities of the corneæ in the individuals * $\frac{Sb\ cr\ In\ (3R)\ Mé}{\text{III Luino Oreg}}$.

Number of individuals 100 for each sex.

Number of irregularities	♀	♂	Total	χ^2
0	86	87	*173*	0·005
>0	14	13	*27*	0·037
Mean	0·18	0·18	...	0·042

Difference 0 ; $\chi^2 = 0{\cdot}042$; $\nu = 1$; P>0·80. Distribution not significantly different.

One sees that in all three characters no significant difference between sexes appears. This is in agreement with the hypothesis of prominent influence of the Y-chromosome.

One possible objection is that the low difference between sexes could be a general effect arising from a new arrangement of different gene systems, produced during the genome substitution.

To remove this objection a second substitution was made between two stocks with high difference between sexes : in this case the expectation according to the Y-chromosome hypothesis is that a high difference should be found.

* These data have been collected by my assistant Dr A. Di Pasquale.

The synthesis of Y and other chromosomes was made using the balanced stocks, as described on page 416.

The stocks chosen were Oregon as Y donor and Crkwenika for the remainder set.

This investigation was limited to the hairs.

Number of individuals 150 for each sex.

Number of hairs .	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*
♀ . . .	4	2	7	11	10	6	6	3	...	...	1
♂ . . .	...	2	3	13	9	9	6	3	3	1	1

Mean ♂ 20·56 Difference 0·84±0·416
Mean ♀ 19·72
The difference is probably significant

One sees that in this case the difference between sexes remained at the same order of magnitude before and after the substitution of chromosomes (before 1·42±0·258, after 0·84±0·416).

C.—A further comparison between the oregonised stocks and the original ones Oregon and Luino is obtained with the following analysis of the variance.

The table summarises the data concerning the comparison for hair frequency of Oregon, Luino and Luino oregonised.

The data are tabulated on pages 418 (Oregon second sample), 421 and 422.

	Oregon		Luino oregonised		Luino	
	♀	♂	♀	♂	♀	♂
Total number of flies .	100	100	45	45	100	100
Means . . .	14·63	17·33	16·70	17·25	21·25	21·83
Sex difference . .	2·70		0·55		0·48	

These data were subjected to an analysis of variance, with partition of the five degrees of freedom of the variance between the six samples into orthogonal components, according to the following scheme :—

Degree of freedom (see text)	Oregon		Luino oregonised		Luino	
	♀	♂	♀	♂	♀	♂
A	+1	+1	−1	−1	...	...
B	−1	−1	−1	−1	+2	+2
C	...	...	+1	−1	−1	+1
D	+2	−2	−1	+1	−1	+1
E	+1	−1	+1	−1	+1	−1

In the table simple coefficients have been indicated. Since there was a difference of the number of individuals per group, the analysis had to be weighted.

In the above partition, E is put in merely to fill the analysis, and tests the significance of the total sex difference, irrespective of stocks. A and B refer to differences between stocks, irrespective of sex difference, A comparing Oregon with Luino oregonised, and B comparing the sum of Oregon and Luino oregonised with Luino. C and D test the significance of sex differences, C comparing the sex difference of Luino with that of Luino oregonised, and D the pooled sex difference of these two stocks to the sex difference of Oregon.

The results of the analysis are then :—

Degree of freedom	Variance = sum of square	*t*	P (per cent.)
A. Difference between Oregon and oregonised Luino stocks	60·42	*3·63*	< 0·01
B. Difference between Luino and pooled Oregon and oregonised Luino stocks	3267·23	*8·47*	0
C. Difference between sex-differences of Luino and oregonised Luino	0·01	*0·002*	> 95
D. Same between Oregon and pooled Luino+ oregonised Luino	133·95	*5·41*	<001
E. Sex difference, total	254·30	*7·46*	0
Total sum of squares between samples .	3715·91	df. 5	...
Variance within samples sum of squares .	2218·26	df. 485	...
Mean square	4·574	...	...

All these variances have one degree of freedom, and hence the *t* test can be used for testing significance. It will be seen that the three stocks differ among themselves, when the sex difference is disregarded and both sexes pooled. However, it is clear that Luino oregonised is nearer to Oregon than to Luino, which fact shows the effect of the

	Oregon		Luino oregonised		Luino	
	♀	♂	♀	♂	♀	♂
Mean . . .	840·90	732·00	801·71	787·63	680·20	704·93
Number . . .	30	30	44	42	30	30
Sex difference . .	+108·90		+14·07		−24·73	

genome on the character. On the other hand, when sex differences are considered, no difference can be observed between Luino and oregonised Luino (comparison C), the only thing in common between the two stocks being the Y-chromosome ; while the sex difference

observed in these two strains taken together is highly significantly different from the sex difference observed in Oregon (comparison D), nd here the only genotypic difference among the first two stocks nd the latter is the Y-chromosome.

The same procedure has been applied to the analysis of corneæ surface, with the previous and following results (data on corneæ, see p. 425) :—

Degree of freedom	Variance = sum of square	*t*	P (per cent.)
A. Difference between Oregon and oregonised Luino stocks	23·50	*0·51*	60
B. Difference between Luino and pooled Oregon and oregonised Luino stocks	4141·46	*6·77*	0
C. Difference between sex differences of Luino and oregonised Luino	9·74	*0·33*	>50
D. Difference between sex difference of Oregon and pooled Luino and oregonised Luino	1426·74	*3·96*	<0·1
E. Sex difference, total	477·70	*2·30*	< 5
Total sum of squares, variance between samples	6079·14	df. 5	...
Variance within samples, mean square .	90·54	df. 200	...

(For this analysis I am indebted to the courtesy of Dr L. Cavalli-Sforza.)

The conclusions which can be drawn from the analysis are that, since the difference between Oregon and oregonised Luino is not significant for the corneæ but significant for the hairs, the first character shows a participation of different parts of the genome beyond the Y-chromosome. What is important is that in both cases the sex difference in Oregon and in oregonised Luino does not show any significant difference.

D.—As a further step to control the conclusions reached from the previous observations, reciprocal crosses were made to test if the presence of a given Y could produce high or low sex difference.

TABLE 4

Heterozygous ♀ from Luino ♀ × Oregon ♂

	I	II	III	IV	V
1	850	735	774	789	934
2	633	962	850	789	740
3	774	881	735	735	716
4	660	711	840	829	885
5	774	945	794	721	779
	738·2	**846·8**	**798·6**	**772·6**	**810·8**

General mean 793·4

The stocks wild Luino and Oregon were chosen, treated with the following technique.

Single pairs of female Luino × male Oregon and *vice versa* were put in culture bottles, and from each culture five individuals were taken to measure the corneæ.

To abbreviate the work, the number of individuals kept low; but to ascertain if from the five cultures a homogenous material has been raised, the data were first submitted to an analysis of the variance, using the F test, as it is shown in the previous and following tables :—

	Sum of squares	Degrees of freedom	Variance
Variance between groups .	39,786·80	20	6,989,34
Variance within groups .	33,305·20	4	8,326·30
Total variance . .	173,092·00	24	7,212·167

$F < 1$.

The difference among the samples is not significant, thus the samples can be pooled.

Heterozygous ♂ from Luino ♀ × Oregon ♂

	I	II	III	IV	V
1	902	669	824	824	669
2	665	581	615	665	633
3	774	814	665	794	611
4	530	886	688	809	611
5	692	834	678	678	568
	712·6	**756·8**	**694·0**	**754·0**	**618·4**

	Sum of squares	Degrees of freedom	Variance
Variance between groups .	193,133·08	20	9,656·65
Variance within groups .	63,696·20	4	15,924·05
Total variance . .	256,829·28	24	10,791·22

$F < 1$.

The difference among the samples is not significant, thus they can be pooled.

The comparison of the mean for males and females, using the *t* test gave the following result (see table 5).

The difference between sexes of heterozygous, where the Y is derived from the Oregon stock, is thus significant.

For the reciprocal cross (female Oregon × male Luino), the same treatment is here summarised (see table 6).

TABLE 5

Surfaces in μ^2 of the corneæ of the heterozygous Oregon ♂ × Luino ♀

♂					♀				
530	615	669	692	824	633	735	774	829	885
568	633	669	774	824	660	735	779	840	934
581	665	678	794	834	711	740	789	850	935
611	665	678	809	886	716	774	789	850	945
611	665	688	814	902	721	774	794	881	962
Total		25					25		
Means		707·400					793·400		

Difference 86·24 ; $t = \frac{86{\cdot}240}{26{\cdot}768} = 3{\cdot}222$; $\nu = 48$; P = 0·001. The difference is significant.

TABLE 6

Heterozygous ♀ from Oregon ♀ × Luino ♂

	I	II	III	IV	V
1	651	651	642	945	609
2	876	669	745	907	824
3	702	724	870	702	814
4	913	789	855	829	745
5	745	730	1009	934	865
	777·4	712·0	824·2	863·4	783·4

General mean 792·2

	Sum of squares	Degrees of freedom	Variance
Variance between groups .	204,035·60	20	10,201·78
Variance within groups .	63,630·40	4	15,907·60
Total variance . .	267,666·00	24	11,152·75

F<1

The differences among the samples are not significant, and the samples can be pooled.

Heterozygous ♂ from Oregon ♀ × Luino ♂

	I	II	III	IV	V
1	871	740	740	735	794
2	1012	716	870	740	870
3	876	678	678	918	907
4	745	716	740	660	907
5	740	721	814	865	789
	848·8	714·2	768·4	783·6	853·4

General mean 793·68

	Sum of squares	Degrees of freedom	Variance
Variance between groups .	132,621·148	20	6,631·05
Variance within groups .	68,312·30	4	17,078·07
Total variance . .	200,933·448	24	8,372·227

$F<1$

The differences among the samples are not significant, and the samples can be pooled.

The measures of the females are then compared with those of the males (see table 7) :—

TABLE 7

Surfaces in μ^2 of the cornea of the heterozygous Luino ♂ × Oregon ♀

♂					♀				
642	702	745	829	907	660	721	740	865	907
651	702	745	855	913	678	735	745	870	907
651	724	789	865	934	678	740	789	870	918
669	730	814	870	945	716	740	794	871	918
669	745	824	876	1009	716	740	814	876	...
Total		25					24		
Means		792·20					793·68		
σ^2		8,372·227					11,152·75		

Difference 1·48

The difference is obviously not significant, thus no further statistical analysis has been made.

The general conclusion which can be reached is that the presence of the Y of Oregon and that of the Y of Luino produce different effect, consistent with the other observations.

5. DISCUSSION

The present investigations give the following information on whether diversities in sex difference are due to the Y-chromosomes or to other parts of the genome :—

(*a*) The sex difference does not change also after substitution of the chromosomes, when the Y-chromosome remains the same.

(*b*) In reciprocal crosses between a stock with high and a stock with low sex difference, when the Y-chromosome is provided by a stock with high sex difference, this remains high, and *vice versa*.

With this evidence, it is possible to discuss the hypothesis put forward in the introduction.

The lack of a significant variation in the sex difference, after a substitution of all chromosomes except the Y, proves that the latter exerts a prominent role in acting on the analysed characters. The maternal effect, *i.e.* the influence of the egg plasma, can be excluded if one considers the results of the reciprocal crosses. In fact, with a maternal effect, the derivation of the egg from Oregon should give a great difference between sexes, and that of Luino a not significant difference ; on the contrary, the individuals coming from eggs from the Oregon stock and carrying the Y from Luino do not show any significant sex difference. It is worth mentioning that no further comparative analysis can be made to exclude any egg influence, because the genotypes : egg Oregon-Y Oregon, egg Oregon-Y Luino, egg Luino-Y Luino, egg Luino-Y Oregon have been obtained in different periods of the investigation, and thus are not suitable for a comparison. We must be content to compare the reciprocal crosses : egg Oregon-Y Luino, egg Luino-Y Oregon, which have been produced in identical conditions and contemporarily and show closely similar figures. It therefore follows that the action of the Y is demonstrated, even when a participation of the egg plasma cannot be denied.

This action of the Y is expressed in not merely one, but three characters, in the same manner. That is, where the difference between sexes is not significant or significant for one character, the same is true for the others.

Obviously this observation leads to consider the genetical composition of the Y-chromosome.

From a general point of view, the following hypotheses can be put forward :—

(*a*) Presence of one gene for each character, all causally acting in the same manner.

(*b*) Presence of only one gene with an apparent pleiotropic activity.

(*c*) Presence of several genes provided of the same type of pleiotropy, building a system of polygenes.

The simultaneous presence of three genes acting in the same manner in the same Y (first hypothesis) seems the most unlikely. The second and the third hypotheses are more acceptable. According to them, the gene should be active in determining an average cell size, irrespective of the kind of tissue. In this case the pleiotropy only should be apparent.

To decide between the second and the third hypothesis (*i.e.* one gene or many genes) further investigations are needed. But also with the data available now, some elements can be found in favour of the hypothesis of polygenes. Above all, the results by Mather (1944) are clearly in favour of the existence of polygenes investigating one character (the sternopleural chetæ) closely similar to those considered in the present paper. Secondly, the behaviour of the stock Oerlikon,

showing a decrease in sex difference during the one year's space recalls a finding already described by Mather. Thirdly, the variety of Y's found is consistent with the hyperthesis of many loci, responsible for the same action.

The possible presence of a system of polygenes in the chromosome acting on the cell size shows a relationship with the Caspersson's theory on the activity of the heterochromatin. If the heterochromatin is the centre where both nucleic acids are synthesised, the cell size must be also controlled by the genetical properties of the heterochromatin. In this view the most different organs may show a common trait dependent on the Y-chromosome genotype, acting at the cellular level. On the other hand those genes, which act on polycellular characters, should be restricted in their control to distinct parts of the body.

This hypothetical view is not disproved by the data obtained from other material.

The influence of heterochromatin on the chaetæ number in *Drosophila* (Mather, 1944) and on pollen-grain life in *Sorghum* (Darlington and Thomas, 1941) are comparable with the present results. The comparison with the variegation of *Drosophila* is less clear, but although not fully understood, it is none the less a disturbance in a character production, limited often to groups of a few cells.

As the present state of the investigation, generalisation seems premature. But, in *Drosophila melanogaster* it is possible to recognise in the heterochromatin of the Y-chromosome an homogeneity in the type of action, which fails to be found in the euchromatic portions of the set.

If further data should be found to give more support to this view expressed now as a mere working hypothesis, heterochromatin might be distinguished from euchromatin as being made up by genes acting in a similar manner on the cell size and other characters of the cell.

To the demonstration of the influence of the Y-chromosome is added the proof of the action of other chromosomes on the investigated characters. The analysis of the variance comparing males and females of Luino and Luino oregonised indicated that the samples are not homogeneous for the wing hairs. This means that, since many parts of the genome are different in the compared groups, these parts (which do not include the Y) are also responsible. It can be concluded that the difference between the mean of each sex indicates the action of the Y, but that the values of the means might be dependent also on other chromosomes.

It must be finally noted that a first approach to the clarification of the genetical determination of the cell size was made as early as 1929 by Dobzhansky, using one of the characters studied in the present investigation, *i.e.* the wing hairs frequency. Although he did not reach any conclusion concerning heterochromatin, his general view is consistent with the theory of a genetical determination of the cell size, independently of chromosome number variations.

6. SUMMARY

1. Three characters have been analysed in *D. melanogaster*: frequency of the unicellular wing hairs, size of the corneæ of the compound eyes, number of the irregularities among the corneæ. All these characters appear dependent on the Y-chromosome as shown by the difference of frequency between sexes. This action on all characters is similar because, where the difference between sexes is significant (or not significant) for one, the same is true for the others.

2. Since all the characters studied are produced by differences of cell size, it seems that the action of the Y-chromosome is primarily exerted on the cell size.

3. An influence on the hair frequency seems proved also for the other parts of the genome, whose actions were, however, less precisely analysed.

4. The findings lead one to consider the heterochromatin of the Y-chromosome mainly as the site of a system of genes (polygenes) acting on small characters detectable only with quantitative analysis.

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GENE DISPERSION IN HETEROGENEOUS POPULATIONS

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In a recent article in *Heredity*, A. J. Bateman (1950) asks "Is gene dispersion normal?" and assembles a considerable body of evidence to support the general rule that diffusionary processes in populations of living organisms give rise to spatial distributions which are leptokurtic.

In discussing the origin of leptokurtosis he states "There is an apparent contradiction between such a situation and the general theory of normal distributions. Under this theory normality should result whenever a large number of uncorrelated influences are at work. The contradiction forces us to the conclusion that neither the passive nor active movement of organisms is at random."

There are, however, alternative explanations which to my mind seem likely. For example, if we consider the positions taken up by the members of a whole population differing among themselves in their activity or powers of dispersion, we can prove—without abandoning the simplifying assumption of randomness—that a leptokurtic distribution must arise of necessity.

For if the position of a particular organism moving randomly in one dimension has probability function

$$f(x) = \exp\{-\tfrac{1}{2}x^2/\sigma^2\}/\sigma\sqrt{(2\pi)}$$

and therefore moment generating function

$$\phi(t) = \exp\{\tfrac{1}{2}\sigma^2 t^2\},$$

and if the value of σ^2 varies between the organisms in any manner whatsoever—say with cumulative distribution function $F(\sigma^2)$—then the probability distribution of the position occupied by an organism chosen at random will have moment generating function

$$\bar{\phi}(t) = \int_0^\infty \exp\{\tfrac{1}{2}\sigma^2 t^2\} dF(\sigma^2) = M(\tfrac{1}{2}t^2)$$

where $M(\tau) = \Sigma \mu_r \tau^r / r\,! = \exp \Sigma \kappa_r \tau^r / r\,!$

is the moment generating function of the distribution of σ^2.

The cumulant generating function of the required distribution is

$$\begin{aligned} \log \bar{\phi}(t) &= \kappa_1(\tfrac{1}{2}t^2) + \tfrac{1}{2}\kappa_2(\tfrac{1}{2}t^2)^2 + \ldots \\ &= \kappa_1 t^2/2\,! + 3\kappa_2 t^4/4\,! + \ldots \quad . \quad . \quad . \quad (1) \end{aligned}$$

Since $\kappa_2 > 0$ by the hypothesis that σ^2 varies, it follows that the 4th

cumulant of this distribution is positive and the distribution leptokurtic by definition.

Looking at this result in reverse we see that the second k-statistic of the observed distribution is an unbiassed estimate of the mean of σ^2 and that $\frac{1}{3}$ the fourth k-statistic of the distribution is an unbiassed estimate of the variance of σ^2. In general

$$\text{Expectation } \{k_{2n} 2^n n\,!/(2n)\,!\} = \kappa_n.$$

Let us now consider the effect of a further period of dispersion on the form of the distribution. For a particular individual the probability distribution will still be normal, and the variance of the final distribution will be the sum of those holding for the two periods separately.

If now the individuals of the population being considered maintain the same *relative* differences in their powers of dispersal during the second period as during the first, the effect of this extension in time is merely to change the scale of σ^2. The value of κ_2/κ_1^2 therefore remains invariant and as a result of (1) the degree of leptokurtosis is unchanged.

If, however, the activities of the individual organisms vary with time depending say on age or physiological condition, then the relative differences between them on any single day will be greater than those displayed on the average over a longer period. In such circumstances the degree of leptokurtosis must decrease in time (as indeed it has been observed to do), though it will not disappear entirely if the individual differences in activity are not completely smoothed out in the long run.

Theoretical distributions capable of graduating data on this subject may be deduced providing that reasonable assumptions are made regarding the variation in the dispersive powers of the individuals.

As an illustration consider the two dimensional case in which the position of a particular organism has density function

$$f(x,y) = \exp\{-(x^2+y^2)/v\}/\pi v$$

where $v = 2\sigma^2$ is the mean square deviation from the origin.

The simple and very reasonable assumption that $w \equiv 1/v$ is distributed among individuals as a gamma variate with density function

$$g(w\,;\,p,\lambda) = e^{-pw} w^{\lambda-1} p^{\lambda}/\Gamma(\lambda)$$

leads to the distribution

$$\bar{f}(x,y) = \int_0^\infty \exp\{-w(x^2+y^2) - pw\} w^{\lambda} p^{\lambda} dw/\pi\Gamma(\lambda)$$
$$= \frac{\lambda}{\pi p} \Big/ \left(1 + \frac{x^2+y^2}{p}\right)^{\lambda+1}.$$

In polar form this distribution is

$$dP(r) = p^{-1} 2\lambda r dr \Big/ \left(1 + \frac{r^2}{p}\right)^{\lambda+1} \quad . \quad . \quad (2)$$

The following relations may then be deduced :—

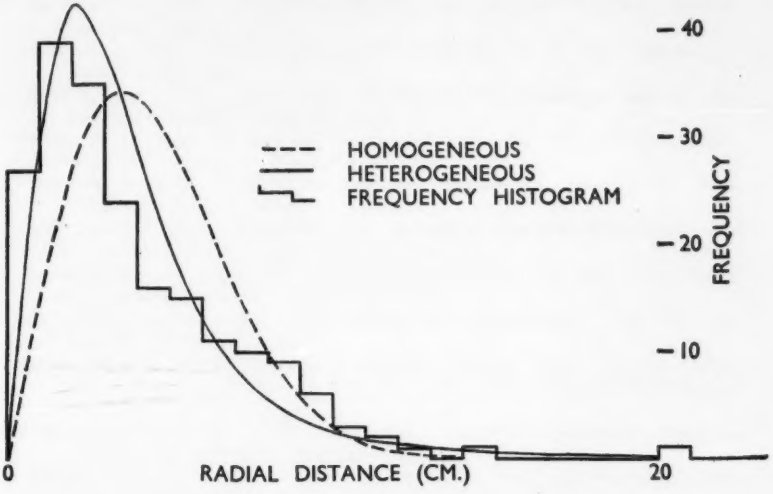
(*a*) The proportion of the population to be expected outside a circle of radius R is

$$\int_R^\infty dP(r) = \left(1 + \frac{R^2}{p}\right)^{-\lambda}$$

(*b*) The mean value of r is $\frac{1}{2}p^{\frac{1}{2}}\pi^{\frac{1}{2}}\Gamma(\lambda - \frac{1}{2})/\Gamma(\lambda)$.

(*c*) The mean value of r^2 is $p/(\lambda - 1)$.

A set of 200 observations on the wanderings of 25 immature millipedes (*Blaniulus guttulatus* Bosc.) taken from the same nest is shown in the fig. The variate r is the radial distance travelled in



cms. in one minute. Function (2) given above is fitted to the data by the method of moments and compared with the function

$$f_0(r) = e^{-r^2/v} 2r/v$$

given by normal theory on the assumption of homogeneity. For this function the maximum likelihood estimate of v is simply the mean square dispersion of the observations from the origin.

In the same way, leptokurtic distributions can be deduced for the expected location of offspring if the parent wanders during the period of its reproductive life.

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BOOKS AND SPECIAL VOLUMES IN THE FIELD OF GENETICS, 1950-1951*

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COMMENT AND REVIEWS

THE MULTIPLE MUTATION IN WHEAT

(1) If, as Lein suggests (*Heredity*, 5, 147-149), varieties of *Triticum vulgare* carry a considerable load of *sv* mutant genes, is it likely that no *sv* phenotypes should have been seen, striking as they are? Yet, as mentioned in my paper, none are on record. The three varieties concerned are respectively of North-Western European, Western Mediterranean, and Canadian (originally Eastern European) origin. Hence *sv* mutations would be either of ancient origin, or of high frequency; one of them is supposed to have arisen in the breeding experiment itself. Why is it then that not in one of the many thousands of wheat crosses made in the last sixty years or more should a *sv* phenotype have occurred either by segregation or by mutation?

(2) On the evidence produced in my paper, the mutant arose as a homozygote. This, according to Lein's assumption, requires not one mutation step, as he suggests, but two, viz., one in each allele of one gene.

(3) The deletion postulated by Lein need not, as suggested in his final sentence, be one of the entire BC arm; unevenly broken bridges supply deletions with a high frequency.

O. H. FRANKEL.

HEREDITARY GENIUS. By Francis Galton. London: Watts & Co. Second edition reprinted 1950. Pp. xxvii+379. 10s. 6d.

Just half a century has now elapsed since the Mendelian renaissance of 1900. The early promise of Mendelian genetics has been richly fulfilled and vast contributions have been made both to our theoretical understanding and to the solution of practical problems. In the sphere of human genetics important applications include the use of blood groups in paternity testing and in predicting the risk of hæmolytic disease of the newborn; also the use of knowledge about the inheritance of a variety of diseases and abnormalities both as an aid to clinical diagnosis and for purposes of eugenic prognosis. It is important to realise, however, that the greatest successes have been obtained with characters which are mainly controlled by single genes, or at most small numbers of genes. Thus the greater part of human genetics is at present concerned with characters whose practical repercussions, though of considerable importance, affect relatively few individuals.

It was Francis Galton who first effectively demonstrated that not only were quantitative characters like stature subject to heredity, but so also were intelligence, fertility and other more subtle and less easily quantified aspects of the human spirit. Galton's use of human data with its small families and doubtful ancestries, together with the emphasis on quantitative characters prevented a satisfactory understanding of the actual laws of inheritance. The statistical approach, initiated by Galton and further developed by Karl Pearson, produced a distinct school of biometricians. There was at first great rivalry and even antagonism between the

biometricians and the Mendelians. Gradually, however, a synthesis was achieved. R. A. Fisher's demonstration in 1918 that the correlation between human relatives could be derived from Mendelian premisses is a notable landmark. Later work in the 1920's and 1930's by Fisher, Haldane and Wright effected a further integration of the two approaches. More recent developments are exemplified by K. Mather's *Biometrical Genetics*, it now being possible to conduct an extensive statistical analysis based on the fundamental concept of a mendelising system of polygenes. If human genetics is to be able to contribute to the framing of general policies of positive eugenics, affecting the whole community, then these studies will have to be further extended to deal with the more insubstantial human characteristics.

In his *Hereditary Genius*, Galton was able to demonstrate that a variety of human faculties *were* inherited and that there were or had been in operation certain social processes, such as the marriage of newly-created peers with heiresses, or the widespread existence of celibacy amongst gifted individuals in the Middle Ages, which were strongly dysgenic in effect. Positive social applications of genetics would deal to a great extent with the kind of material discussed so clearly and brilliantly by Galton. It is to be hoped that this re-issue of *Hereditary Genius*, which contains much that is still interesting and pertinent, will act as a stimulus to new studies of Man using the methods of biometrical genetics now available. If it should be possible to re-work Galton's approach with modern techniques, we might expect this to have far-reaching consequences for the improvement of the human stock.

It goes without saying that Galton was subject to the limitations of his time, and this is scarcely worth dwelling on in detail. One important point is, however, worth mentioning. Galton makes extensive use of published biographies of eminent men, and does so with great discrimination and ability, but on the whole accepts them as they stand. A modern discussion of similar material would attempt to allow for the complication of ascertainment, which has been dealt with quite successfully in other problems. Families with many gifted individuals are more likely to be noticed and to find their way into the record and this will preclude a simple interpretation of the data. Some kind of artificial selection of this sort may well be responsible for the apparent " anticipation " involved in the view that " the sons of gifted men are decidedly more precocious than their parents "—a statement which is not easily reconciled with Galton's own studies on the " regression to the mean."

As Galton himself wrote in the conclusion to the Preface to the 2nd edition of 1892, " I wish again to emphasize the fact that the improvement of the natural gifts of future generations of the human race is largely, though indirectly under control. . . . It is earnestly to be hoped that inquiries will be increasingly directed into historical facts, with the view of estimating the possible effects of political action in the future, in gradually raising the present miserably low standard of the human race to one in which the Utopias in the dreamland of philanthropists may become practical possibilities."

NORMAN T. J. BAILEY.

GENETICS AND THE RACES OF MAN : An Introduction to Modern Physical Anthropology. By W. C. Boyd. Oxford : Blackwell. 1950. Pp. xvii+453. 30s.

As indicated by Professor Boyd in his subtitle to *Genetics and the Races of Man*, this book is intended specially for students of physical anthropology. The need for a textbook supplying anthropologists with a genetical background for their investigations on the measurement of man has long been evident. Let it be said at once that Professor Boyd's book supplies this want in a satisfactory manner.

Professor Boyd's approach to the subject which is set forth in the first chapter, is that anthropology without racial prejudice is possible and, indeed, necessary. In scientific enquiry no peoples are superior or inferior to others ; they are just different from one another. The careful introduction to genetics which follows is suitable for students acquainted only with the elements of biology. Ideas, which are especially important in human genetics, such as the interaction of heredity and environment, gene frequency, equilibrium in the presence of natural selection and " drift," are competently discussed. Although there is not as yet general agreement upon many of the points raised it is advantageous to stimulate biological and genetical thinking among anthropologists by bringing these problems to their notice. The adaptive value of skin colour is stated as a fact ; this is perhaps reasonable but the adaptive value of dwarf populations where food supply is limited is more dubious. Race can be defined as a " constellation of characters " and in many other ways. In view of his admission that the original human strains cannot now be identified it would have been more logical for the author to have advocated dropping the word " race " altogether. Description of populations in terms of gene frequencies, mean cephalic indices or mean skin colours, is quite objective. The retention of the word " race " with its taxonomic implications, which Professor Boyd discards as unscientific, even if a new definition is provided, seems quite unnecessary.

A great deal of well-tabulated factual information is provided in the book. It is not quite up to date, but that will not seriously affect the value of the book in the sphere where it is intended to be used. A number of useful statistical methods are described in appendices but, in discussing the coefficient of racial likeness, and in pointing out its deficiences, no reference is made to Zaraphin's very convenient measure. This coefficient is equivalent to the variance of a set of standardised character differences between populations and it has been successfully used by Cavalli. The account of technique of taste testing is quite inadequate and the reader will look elsewhere for the very latest results of blood grouping of populations with the newer antigens. Little attention has been given to the question of the distribution of genes for inherited diseases or metabolic anomalies, such as sickle cell trait, thalassæmia, phenylketonuria and xyloketosuria which are known to have very marked local frequency differences. Moreover the table of Mendelian characters in man, pp. 318-320, is so full of flagrant errors that it could best be described as an erratum for immediate and total deletion. For a textbook of over 400 pages on a subject not previously treated in this manner the number of misleading statements included is not large and the reader's interest is maintained throughout.

L. S. PENROSE.

GENETICS IN OPHTHALMOLOGY. By Arnold Sorsby. London: Butterworth & Co. (Publishers) Ltd. 1951. Pp. xi+251. 42s.

The scientist may consider that this book lacks precision, that ill-defined concepts are discussed on many pages, and that others are occupied by illustrations of clinical conditions which are incomprehensible to him. It is true that nowhere in the test is there a clear account of the present human situation. There was a time when it might be truthfully said that ophthalmological genetics was the most highly developed branch of medical genetics, but this is no longer the case. This honour now clearly belongs to the serologists, and although the blood-group systems are referred to briefly, the fact that at least nine of the twenty-four human chromosome pairs are identifiable is not mentioned.

The introductory chapters are too brief and too complex for the clinician to derive much benefit from them. The books by Ford or Fraser Roberts give a very much better introduction because they do not attempt so much. It is doubtful if there are many medical men who would find the following paragraph comprehensible without much more detail than is contained in the context.

> "A gene is so subject to total genic balance that the conception of dominance and recessiveness serves well for particular individuals or generations only, but not as an absolute measure. A gene, by itself, is neither recessive nor dominant. These states are the result of the interaction with the other genes and with environmental factors. Under experimental conditions one and the same gene in two different genetic environments may be either recessive or dominant. There are good grounds for believing that a mutant gene is frequently dominant on its first appearance, but becomes either completely eliminated or steadily more recessive as the accumulation of modifying factors by natural selection aids the necessary conditions which prevent the dominant mutant from expressing itself. The gene reaches full recessiveness when it can express itself only in the duplex state."

The problem of linkage is not made very clear and the medical reader might easily suppose that when two genes are linked there is a tendency for the particular alleles to stick together in subsequent generations. The fact that they occur separately as often as they do together in the population, and that this is an essential feature of linkage, does not emerge from the text. There is no reference to the extremely valuable work carried out by Hoogvliet, who published ten pedigrees segregating for both hæmophilia and colour blindness. In one of these it was possible to give sensible advice by using the relatively innocuous factor producing colour blindness as a marker for the more serious hæmophilic gene. This work carried on the best traditions of Nettleship and Usher. This cannot be said for all the examples given in the book.

There are several tantalising sentences to be found throughout the text. For example "The advice that would be given on the assumption that the patient suffers from the common form of recessive retinitis pigmentosa would be completely wrong, and disastrous, if it should prove that the patient's affection is transmitted in a dominant or a sex-linked manner." The reader is not told the advice to give.

It is still the first duty of the clinician, wishing to enrich our knowledge

of hereditary disease, to present his findings as objectively as possible. Factual knowledge is still scarce and theoretical speculations arising upon foundations of incomplete data must be treated with care, or indeed rejected outright. In the past clinicians were frequently criticised for a tendency to publish only those families which showed well-marked inherited anomalies, with the result that isolated examples tended to be neglected, and did not appear in the literature to the expected extent. The danger now developing is that inadequately worked out pedigrees will be placed on record in order to illustrate some preconceived theoretical situation, for example partial sex-linkage.

Abiotrophy is a term introduced by Sir William Gowers about fifty years ago to signify degeneration of tissues due to defective vitality. Hereditary optic atrophy, retinitis pigmentosa and certain forms of macular degeneration may be cited as examples. The author of this book has devoted much care and attention to the abiotrophic character of many genetic anomalies affecting the eye. He believes that it is not unlikely that some of the so-called senile degenerations are late abiotrophic manifestations, and that evidence is accumulating to show that senile cataract and primary glaucoma are essentially genetic affections.

The book is written with the zeal and enthusiasm of the advocate and the teacher. It is intended to arouse the interest of the clinical worker and it should attain this objective with ease. The selected bibliography is arranged at the end and follows the textual sequence. This makes for ease in reference and is a practice which might well be used more extensively. The illustrations and production are an attractive feature of the book.

W. J. B. RIDDELL.

METHUEN MONOGRAPH : The Measurement of Linkage in Heredity. By K. Mather, D.Sc., F.R.S. Second edition. London : Methuen. 1951.

It is very satisfactory that Professor Mather's small but closely packed monograph is again in print. The first edition was the only textbook entirely and explicitly devoted to the design of laboratory investigations in genetics and their statistical treatment, and it filled this role with distinction. Its especial merit lay in the successful fusion of two different and almost contradictory aspects. In the first place it had the character of a Laboratory Handbook in which designs of experiments (together with formulæ and computational procedures needed for their assessment) were provided "ready-made" (or in easily adaptable form) for the assistance of the research worker in a variety of situations. In addition, however, the principles of design and statistical interpretation were developed by particularly clear and simple reasoning in such a way as to bring out the reader's statistical horse-sense. This made it a book of exceptional value for the training of geneticists whether recruited from mathematics or biology. A mathematician of my acquaintance says that he first learnt statistics from it; I myself was, and still am grateful for it, and I know it to have been of constant utility to many non-mathematical colleagues.

In the second edition the original text has been retained (with some extensions) so that the value of the monograph is undiminished. Some misprints should be noted which have survived without correction from the first edition: On page 100, 1936*a* should read 1949*a*. The item $(1+y'+2yy')$ appearing in the formula at the top of page 109 (and on

page 139) should read $\{1+y'+(s-3)yy'\}$. On page 124, a paragraph is duplicated. The item $S\{t(9-1)n_{at}\}$ on page 139 should be $S\{t(a-1)n_{at}\}$. On page 100, the author might profitably have added the remark that maximum precision is attained when the products A_{R_1} A_{R_2} and A_{C_1} A_{C_2} are brought most nearly to equality.

There are some major additions to the text, of which a very useful one is a section on Fisher's scoring method. Here it may be felt that the author has perhaps obscured a particular merit of this method by choosing in his worked example to calculate the information algebraically instead of numerically as score increment divided by difference in parameter values, despite the fact that he has already indicated the possibility of this procedure in an earlier section of Chapter VII. It is perhaps to be regretted as a lost opportunity that the whole of this chapter was not revised in order to make the arithmetical advantages and mental economy of scoring technique more explicit. None the less this chapter still holds its place as one of considerable utility.

The other important addition which the author has made, is a wholly admirable chapter on the estimation of population gene frequencies. Here the author shows very effectively how to exploit partitioned χ^2, and gives a full account of the modern technique of estimation when three alleles are involved. A. R. G. OWEN.

THE PRINCIPLES OF HEREDITY. By L. H. Snyder. Fourth edition 1951. Boston : D. C. Heath & Co. $4.50.

The principles of heredity arise from the experiments and ideas of Mendel and Weismann, Darwin and Galton, Bateson and Morgan. The growth and nature of these principles are not widely known and the experts have actually been confused about them. Their confusion does not seem to detract from the value of the research work they do. It has, however, certain concealed consequences. It limits their outlook. It prevents genetics from attaining its natural scope and new genetic discoveries from being understood by those who might be expected to understand them.

Professor Snyder's *Principles of Heredity* is intended to introduce " beginning students " to genetics. It derives the principles to which its title refers from Mendel and Morgan. It avoids certain complications by omitting Darwin and Weismann and dismissing Bateson as one of the joint discoverers of linkage.

How Professor Snyder treats the legacy of Mendel and Morgan may be seen from one parenthetical sentence (p. 17). " Mendel did not use the word ' gene ' . . . his hypothetical ' factors ' behaved as today we know genes to behave. We shall, therefore, use ' gene ' in relating Mendel's work." In other words, if Mendel had used the word " factor " he would have meant what Professor Snyder means by the word " gene." Some may think that there is nothing wrong in introducing the word gene into a description of Mendel's experiments. Some may feel also that the word gene is so familiar to all of us that it requires no definition. But in fact Mendel spoke only of " characters," " series," " cells " and " elements " and Bateson spoke of " unit-characters " (without defining them) only later being driven to assume " determiners " and " factors " by the consideration of dominance, presence and absence, and interaction. And surely this is of some importance in understanding the great assumptions of principle

involved in genetics. If the beginning student is given no idea of the difficulties there have been—and still are—in bridging the gap between character and determinant, he can scarcely know what the principles of heredity are about.

Passing now to a later period, in the last thirty years the great advance in the principles of heredity has been in understanding the mechanism of crossing-over, its universality, its relation to the theory and practice of recombination, and to the developing notion of the gene. On all of these things Professor Snyder dithers. He does not seem himself to understand how or when crossing-over takes place. Nor does he make clear how the event of crossing-over and the idea of the gene are connected—or even what he means by a gene, beyond saying that Mendel did not use the word.

On this foundation Professor Snyder builds a theory of human variation and evolution of the most advanced type under the title of "The mutant gene in man." He proceeds with abundant mathematics (taking, let us hope, the beginning student with him) but still without any precise use of words. The conclusion he reaches is therefore emphatic without being exact :—

> "It can be shown, in fact, that in populations numbering a few dozen or score of mating individuals, *the direction of gene-frequency shifts at any locus is in large part independent of the relative selective values of the alleles ; unless the intensity of selection is quite high, fixation or extinction of an allele depends almost exclusively on genetic drift . . .*" (p. 430).

These sentences have, we must suppose, no relevance to man. Neither, however, have they any definite meaning. They convey an aspiration ; and in this respect perhaps they epitomise Professor Snyder's book.

The Principles of Heredity has 155 delightful illustrations. It will no doubt enable the readers of the 250 colleges in which it is used to answer the 505 problem questions set out for them. The text deals with every aspect of the technology of genetics ; a subject to which a great many books have been, and will be, devoted. But it does not introduce the beginner or anyone else to the principles of the subject or to the methods of inquiry on which those principles rest. And in genetics, if he wishes to go far, the beginner will find that principles matter. C. D. DARLINGTON.

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